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GP 1814

Attorney's Docket No. TxTox Inc.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: McDaniel, et al.

Serial No.:	08/252,384	Group No.:	~~1814~~
Filed:	June 1, 1994	Examiner:	C. Low
For:	Recombinant Organophosphorus Acid Anhydrase and Methods of Use		

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

TRANSMITTAL OF APPEAL BRIEF (PATENT APPLICATION—37 CFR 192)

1. Transmitted herewith in triplicate is the APPEAL BRIEF in this application with respect to the Notice of Appeal filed on November 25, 1994.

NOTE: "The applicant shall, within 2 months from the date of the notice of appeal under § 1.191 in an application, reissue application, or patent under reexamination, or within the time allowed for response to the action appealed from, if such time is later, file a brief in triplicate. 37 CFR 1.192(a) [emphasis added].

2. STATUS OF APPLICANT

This application is on behalf of

☐ other than a small entity.

☒ small entity — verified statement:

- ☐ attached.
- ☐ already filed.

3. FEE FOR FILING APPEAL BRIEF

Pursuant to 37 CFR 1.17(f), the fee for filing the Appeal Brief is:

☒	small entity	$140.00
☐	other than a small entity	$280.00

Appeal Brief fee due $ 140.00

CERTIFICATE OF MAILING/TRANSMISSION (37 CFR 1.8(a))

I hereby certify that this correspondence is, on the date shown below, being:

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☒ deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

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Date: 4-25-95

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Signature

C. Steven McDaniel

(type or print name of person certifying)

4. EXTENSION OF TERM

NOTE: The time periods set forth in 37 CFR 1.192(a) are subject to the provision of § 1.136 for patent applications. 37 CFR 1.191(d). Also see Notice of November 5, 1985 (1060 O.G. 27).

(complete (a) or (b) as applicable)

(a) ☒ Applicant petitions for an extension of time under 37 CFR 1.136 (fees: 37 CFR 1.17(a)-(d) for the total number of months checked below:

	Extension (months)	Fee for other than small entity	Fee for small entity
☐	one month	$ 110.00	$ 55.00
☐	two months	$ 370.00	$185.00
☒	three months	$ 870.00	$435.00
☐	four months	$1,360.00	$680.00

Fee: $435.00

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item, if applicable)

☐ An extension for _____ months has already been secured and the fee paid therefor of $ _____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request: $ _____

OR

(b) ☐ Applicant believes that no extension of time is required. However, this conditional petition is being made to provide for the possibility that Applicant has inadvertently overlooked the need for a petition for extension of time.

5. TOTAL FEE DUE

The total fee due is:

Appeal brief fee $ 140.00
Extension fee (if any) $ 435.00

TOTAL FEE DUE: $ 575.00

6. FEE PAYMENT

☒ Attached is a check in the sum of $575.00.
☐ Charge Account No. 03-2769 the sum of $_____.

A duplicate of this transmittal is attached.

7. FEE DEFICIENCY

NOTE: If there is a fee deficiency and there is no authorization to charge an account, additional fees are necessary to cover the additional time consumed in making up the original deficiency. If the maximum, six-month period has expired before the deficiency is noted and corrected, the application is held abandoned. In those instances where authorization to charge is included, processing delays are encountered in returning the papers to the PTO Finance Branch in order to apply these charges prior to action on the cases. Authorization to charge the deposit account for any fee deficiency should be checked. See the Notice of April 7, 1986, (1065 O.G. 31-33).

6. ☒ If any additional extension and/or fee is required, this is a request therefor and to charge Account No. 03-2769.

AND/OR

☐ If any additional fee for claims is required, charge Account No. 03-2769.

SIGNATURE OF ATTORNEY

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GP 1814

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	**McDaniel, et al.**	§	
		§	
		§	**GROUP ART UNIT: 1814**
SERIAL NO.:	**08/252,384**	§	
		§	**EXAMINER: C. Low**
FILED:	**June 1, 1994**	§	
		§	
FOR:	**Recombinant Organophosphorus Acid Anhydrase and Methods of Use**	§	

Atty. Dkt. No. TXTox, Inc.
Date: April 25, 1995

APPLICANT'S APPEAL BRIEF

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APPLICANT'S APPEAL BRIEF

INTRODUCTION

This is an appeal of the Examiner's final rejection mailed August 24, 1994, of the claims pending in the above referenced case. The Board of Patent Appeals and Interferences is respectfully requested to consider Appellant's arguments and to reverse the final rejection of the claims.

STATUS OF CLAIMS

Claim-By-Claim Status

Claims 53-64, 67 and 68 are pending in the case. Claims 53-64 stand rejected. Claims 67 and 68 remain withdrawn by the Examiner's requirement for restriction in the Office Action mailed 6 December 1993 (Paper No. 17 of parent application serial number 07/928,540).

Claims on Appeal

The rejection of claims 53-64 is appealed.

STATUS OF AMENDMENTS

Amendments Filed Subsequent to Final Rejection

No amendments have been requested in the case subsequent to the final rejection.

Status of Amendments

No amendments have been entered into the case subsequent to the final rejection.

SUMMARY OF INVENTION

Organophosphorus compounds ("OPs") have been designed which are highly toxic to the human nervous system. They are one of the principal categories of nerve gases. They were, in fact, the evil seed spawned out of the concentration camps of war, and have horribly killed many thousands of innocent people. One need only recall the recent Japanese subway terrorist

attacks using the OP nerve gas sarin, or Saddam Hussein's threat to use OP nerve agents against U.S. troops in Operation Desert Storm and his actual use of these materials against the Kurdish rebels, to realize that the threat of these compounds remains real.

This is to say nothing of the pressing need to rid the United States and other countries of the enormous stockpiles of deteriorating OP nerve gases. Perhaps even more importantly, it is nothing to say of the growing worldwide concern over the use of OP compounds as pesticides in agriculture and in millions of homes, such as parathion, malathion, diazinon, dursban, chlorpyrifos and the like.[1]

Prior to the present invention, there were very few means for treating such toxic compounds. All such "cures" were almost as toxic and undesirable as was the "illness." Concentrated solutions of caustics and incineration, were and still are, the methods used. However, as the recent U.S. Department of Defense experience illustrate, the populace surrounding sites at which caustics or incineration are used to destroy unwanted OP toxins are rigid in their resistance to such techniques being used in their communities. Exhibit A. Alternatives to incineration and caustic hydrolysis are needed . . . the OP nerve gas stockpiles continue to dangerously deteriorate . . . the threat of chemical warfare is ever with us and OP toxic compounds appear to provide third world powers and terrorists with a weapon of fear . . . people are exposed everyday in their foods and in their homes to OP pesticides with no simple way to ensure that they are protected.

The present invention provides an alternative. It may not be the cure-all. It may not work in every instance. But, it is a viable alternative to the methods presently used which have many undesirable characteristics. There is truly long-felt and unfulfilled need for commercial

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[1]The Board is respectfully requested to review a short (less than 2.0 minutes) news report broadcast on ABC's *World News Tonight* (5/9/91) entitled "Safety of Lawn Pesticides" (a transcript is provided as Exhibit C) which reenforces Appellant's arguments that there is a long-felt and unfulfilled need to create alternatives to and methods of treating OP pesticides. A similarly supporting news broadcast was run on *NBC Today* (5/16/91) for which a transcript is provid d, with relevant pages 26-28 (Exhibit D).

products capable of detoxifying OP toxins without resorting to caustic chemicals and incineration. Short of the commercial incentive afforded by patent protection, it is not likely that the alternatives provided by the present invention will be commercialized.

Prior to the present invention, it was known that microorganisms could degrade certain organophosphorus compounds used as pesticides. It was also known that certain bacteria exhibited a particular penchant for doing so. At least certain bacteria appeared to be able to attack a wide array of OPs. It was not known if the activity exhibited was fully or partially enzymatic in nature, since the OPs were so lipid-soluble. More importantly, it was nor known, if in fact the degradation of OPs exhibited by the bacteria was enzymatic, or whether there was one or a multitude of enzymes responsible for the activity. It was seriously questioned whether such enzymes would be capable of breaking down the more recalcitrant of the OPs such as those with P-S linkages, including certain OP nerve gases.

Attempts had been made to isolate the enzyme or enzymes from certain of the bacteria, but only crude extracts could be made. The enzyme or enzymes appeared to be intractably linked with the cell membrane. Without the ability to get at least small portions of a purified enzyme, the Appellants and others struggled with ways to get a handle on the gene. Not the least of their problems stemmed from that fact that the bacteria in which the greatest activities were found were soil isolates which were not fastidious under laboratory conditions. *See*, e.g., specification at page 5, lines 4-5. Even more problematic was the total lack of selective plate assays which could distinguish colonies of bacteria expressing the activities the Appellants sought. *See*, e.g., specification page 4, lines 23-26.

It was not known where in the bacterial genome the genes encoding these enzymes would be found. It was not known whether the enzyme or enzymes could be expressed in another cell other than the ones in which they originated.

It was not known whether the enzyme or enzymes, even if the genes encoding them could be isolated and expressed, would exhibit the desired activities. It was suspected that the enzyme

or enzymes were membrane-bound and might require membrane fragments to even work. There was no way to predict if the enzymes when synthesized in heterologous bacteria, would retain their activity, especially where the membrane systems were distinct. Even more so, there was no way to predict whether the bacterial enzymes would be active in the distinctive environment of a eukaryotic cell system. It was not known if such isolated, recombinant enzymes would exhibit single or multiple OP activities. It was not known if such recombinant enzymes would be capable of attacking the more noxious of the OPs, the OP nerve agents.

Standard laboratory procedures for isolating the DNA of these soil bacteria were unsuccessful and, in particular, the large plasmid DNA of these bacteria turned out to be very difficult to isolate in a condition in which it was useful. *See*, e.g., specification at page 4, lines 32-34. Even after the inventors developed successful techniques for the isolation of the plasmid DNA from these soil microbes, considerable designing of experiments was required in order to produce successful expression hook-ups -- a fact strengthened in its relevance by the previous failure of the inventors as well as several other groups to achieve acceptable levels of expression in any but the original strains.

One of the most difficult of the roadblocks which the inventors had to overcome involved the accurate sequencing of the DNA from these soil bacteria. This procedure proved to be exceedingly difficult due to the high ratio of G-C to A-T in these particular bacteria. In fact, these bacteria are known to have one of the highest such ratios among all bacterial genera.

Even more difficult for the inventors was the task of producing enough of the membrane-associated enzyme in order to purify and characterize it. *See*, e.g., specification at page 5, lines 1-3, and 5-7. Until this was achieved, there was no way by which to verify the actual coding sequence for the enzyme and thus no way to accurately design hook-ups which allowed for expression in the variety of hosts. *See*, e.g., specification page 8, lines 28-32. This was especially true since this enzyme is most likely cleaved of an N-terminal signal sequence prior to insertion into the native host bacterial cell membrane. Thus, only by virtue of the knowledge gained in the successful expression of the bacterial enzyme and knowledge of its complete

sequence were the inventors able to design vectors for the use with a eukaryotic expression system. It is noteworthy in this regard to observe that although minor modifications to the sequence have been made by the inventors and others since the present patent application was filed, there have been no scholarly disagreements concerning the originally identified N-terminal sequence of the opd gene.

The inventors and other failed in achieving the quantities of enzyme necessary for substantial testing or use of the enzyme from the native bacterial sources. *See*, specification at page 8, lines 34-36 through page 10, lines 1-9. High level expression of the enzyme behind promoters known in bacteria to produce large amounts of other enzymes did not prove suitable. Likewise, there was no way of knowing whether the membrane-associated enzyme from soil bacteria would be expressed to any useful degree by eukaryotic host cells. Surprisingly, however, manifold levels of enzyme were produced allowing for the purification and characterization of the enzyme by specialized eukaryotic cells selected by the inventors which had eluded the inventors and others for years. *See*, e.g., specification at page 10, lines 11-19.

Once the enzyme and the gene encoding the enzyme were capable of being manipulated by virtue of the present invention, a wide range of uses became possible. Among these uses are: commercial-scale detoxification of organophosphorus compounds in vitro; detection of organophosphorus compounds; protection of susceptible organisms, including humans and beneficial insects, from organophosphorus poisoning; detection of organophosphorus-detoxifying microorganisms; environmentally-sound pesticide design and controlled detoxification; nerve gas detoxification; among others.

The prior art failures and the present invention successes are particularly noteworthy because of the following achievements: (1) a complete, and essentially correct, DNA sequence; (2) a determination of the coding sequence and, in particular, the determination of the correct start codon and reading frame within the sequence; (3) heterologous expression of the enzyme in bacteria at easily detectable levels; (4) heterologous expression of the enzyme in eukaryotic cells; (5) isolation of and purification of utilizable quantities of the recombinant enzyme; and,

(6) use of the knowledge gained by the inventors to design successful hook-ups allowing expression and transformation of multi-cellular animals.

Many had tried to achieve what the Appellants eventually did achieve. But, it was the Appellants who, using a novel approach, unexpectedly and surprisingly isolated the gene encoding the enzyme of interest in a compact complete genetic fragment without excessive superfluous sequence, expressed it in a number of recombinant hosts including eukaryotes, and proved that the single enzyme was responsible for not only detoxification of pesticides, but also detoxification of nerve gases.

Reading the rejected claims on the disclosed invention shows that several methods of using the recombinant detoxification enzyme have been disclosed. Claim 53 relates to a method for detoxifying an OP which comprises exposing an OP to the recombinant bacterial organophosphorus acid anhydrase (OPH). Specification, p. 13, l. 15, *et seq.* As noted in the specification, detoxification is achieved by causing a hydrolytic reaction to occur across the susceptible bond of the OP compound. Specification, p. 13, l. 20-22. By way of example, the detoxification of the OP parathion is described. Specification, p. 13, l. 22-25.

Claim 54 relates to a refinement of the method of claim 54 on which it depends applying the method to a matrix comprising the OPH enzyme. Specification, p. 13, l. 30-34. In this embodiment, either the recombinant OPH enzyme or a recombinant cell is attached to a matrix allowing the construction of a column over which the OP-containing substance may be passed. As noted, when a applied directly to specific situations, the embodiment of claim 54 may be used in a filtration scheme as claimed in claim 55 (specification, p. 13, l. 34 - p. 14, l. 2) or in a gas mask as claimed in claim 56 (specification, p. 14, l. 2-7). When used in such embodiments, the recombinant enzyme or recombinant microorganisms expressing the enzyme can be exposed variously to either air (claim 57; specification, p. 14, l. 7-11) or a fluid (claim 58; specification, p. 14, l. 11-16).

Claim 59 relates to an embodiment in which the recombinant OPH enzyme is used to

spray onto a locus comprising toxic OP compounds for purposes of detoxifying them. Specification, p. 14, l. 18-31. Claim 60 is an embodiment in which the recombinant OPH enzyme is introduced into a contained locus exhibiting OP contamination such as a spent commercial-scale pesticide or military nerve gas canister, a military vehicle, a home pesticide bottle or can, or even in the contained environment of a human or animal gut following exposure to OP poisons. Specification, p. 14, l. 33 - p. 15, l. 1-10. Of course, where prevention of toxicity is desired, as in the embodiment claimed in claim 64, a locus may be pre-treated with the recombinant OPH enzyme or microorganism.

Claim 61 further refines claim 53 by providing for the gene encoding the OPH enzyme to be produced in a transformed microorganism when operably linked to an expression vector. Claim 61 recites that the gene of interest will have a particular gene sequence, and that sequence is specifically recited in the claim. The sequence is the same as that shown in Figure 1 of the specification. (The Board is reminded that this application was filed prior to the sequence disclosure rules were put in place). The types of vectors (and promoters) and types of microorganisms provided by way of example illustrate the approach taken. Specification, Table 1, p. 9. The same approach can be seen when, as in claim 63, a transgenic eukaryotic organism is used as the expression system. Specification, Table 1, p. 9.

ISSUES

I. Is Restriction to Claims 53-64 Proper?

II. Have Appellants Complied With 37 C.F.R. 1.52(c), 1.56 and 1.67(a)?

III. Have Appellants Used Trademarks Inappropriately?

IV. Does the Specification Provide a Reasonable Written Description for Practicing the Claimed Invention, and Are the Claims Properly Rejected Therefor?

V. Is the Disclosure Enabling Only for Claims Limited to The Specifically Disclosed Compounds Such as Parathion, Paraoxon, and Methyl Parathion?

VI. Are the Claims Indefinite for Failing to Particularly Point Out and Distinctly Claim the Subject Matter Which Appellant Regards as the Invention?

VII. Are Claims 53, 54, 58, and 59-63 Anticipated by McDaniel *et al.*?

VIII. Are Claims 53, 54, 58, and 59-63 Obvious Over McDaniel *et al.*?

IX. Are Claims 53, 54, 58, and 59-63 Anticipated By Harper *et al.*?

X. Are Claims 53, 54, 58, and 59-63 Obvious Over Harper *et al.*?

XI. Are Claims 53, 58, and 60 Anticipated By Wild *et al.*?

XII. Are Claims 61-63 Obvious Over Wild *et al.*?

XIII. Are Claims 53, 54, and 60 Anticipated By McDaniel?

XIV. Are Claims 61-63 Anticipated By McDaniel?

XV. Are Claims 61-63 Obvious Over McDaniel?

XVI. Are Claims 53-54 and 59-64 Obvious Over Munnecke I, Taken With Munnecke II, McDaniel *et al.*, and Gottlieb?

XVII. Are Claims 53-54 and 59-64 Obvious Over Munnecke I Taken With Munnecke II, Wild *et al.*, and Gottlieb?

XVIII. Are Claims 55-57 Obvious Over Munnecke I Taken With Munnecke II, McDaniel *et al.*, and Gottlieb, or Obvious Over Munnecke I Taken With Munnecke II, Wild *et al.*, and Gottlieb As Applied To Claims 53-54 and 59-64, and Further In View of Grot *et al.*?

GROUPING OF CLAIMS

Claims 53-64 are all properly of a single group.

ARGUMENT

(i) Rejections Under 35 U.S.C. § 112, First Paragraph

ISSUE IV: Does the Specification Provide a Reasonable Written Description for Practicing the Claimed Invention, and Are the Claims Properly Rejected Therefor?

YES. DISCLOSURE OF THE MOST ACCURATE DNA SEQUENCE KNOW TO THE APPELLANTS AT THE TIME OF FILING, WHERE THE SEQUENCE DISCLOSED IS INHERENT IN THE FRAGMENT

SPECIFICALLY DESCRIBED AND FULLY ENABLED, PROVIDES THE MOST REASONABLE WRITTEN DESCRIPTION FOR PRACTICING THE CLAIMED INVENTION

A. The Examiner's Objection

The Examiner has objected to the specification under 35 U.S.C. § 112, first paragraph, for failing to provide a reasonable written descriptio for practicing the claimed invention. In particular, the Examiner has pointed out that the specification recited using P. diminuta and a Flavobacterium sp. (ATCC 27551) (the Examiner calls attention to Harper et al., BX , and McDaniel et al., BY) which the Examiner states set forth DNA sequences coding for opd where the organophosphorus acid anhydrase DNA set forth in Figure 1 of the specification are only partially identical. The Examiner takes the position that, from the recited examples in the specification, it is not readily apparent that the species of bacteria are any different, that the plasmids used are any different, that the isolated DNA that was sequenced was any different, or that the functionality encoded by the DNA is any different. Yet, according to the Examiner, the sequences recited in the Harper et al., the McDaniel et al., the Mulbry et al., and Figure 1 of the specification set forth different DNA sequences coding for what is apparently the same enzyme.

The Examiner further notes that the specification recited using the plasmid pCMS1 (Fig. 2 of Harper et al.) and sets forth the DNA sequence (Fig. 1). The Examiner argues that this is apparently the same plasmid and DNA in the specification and the RESULTS section of the McDaniel et al., reference. The Examiner cites in support of this conclusion that Fig. 4 of the McDaniel reference is identical to Fig.2 of the present application.

In conclusion, the Examiner takes the position that there are apparently at least three different references all directed to the apparently identical genetic material where no reference indicates a sequence identity for the apparently identical genetic material. Therefore, according to the Examiner, a query is raised as to what genetic material is disclosed as having the properties of the organophosphorus anhydrase.

B. Appellants' Remarks

Appellants urge that by following the teaching of the patent application as submitted, one of ordinary skill in the art may obtain, without undue experimentation, the PstI fragment represented in Figure 1 of the patent specification. As pointed out int eh specification, a source of the DNA amenable to the teachings of the present invention is deposited with the ATCC as Flavobacterium sp. ATCC No. 27551 (specification p. 7, lines 2-29). Furthermore, in order to utilize the fragment in any of the embodiments of the invention, one of skill in the art will realize that the most important sequence information provided by the specification in Figure 1 is the ATG start site identified and verified by the inventors only after considerable effort and invention. IT is important to note that no modifications to the sequence which have been suggested by the inventors or others modify any portion of the region surrounding the originally identified start site which site and immediately surrounding sequence is critical in the preparation of proper hook-ups for expression of the gene. For instance,Serdar et al. (Bio/Technology 7:1151-1155 (1989)) suggests a change in the sequence of Figure 1 of the present application at a site no closer than 55 base pairs upstream of the start site identified by the inventors, while Mulbry et al. suggests a change at a site no closer than 35 base pairs from the same ATG start site.

Thus, it is submitted that Figure 1 of the present specification complies with 35 U.S.C. § 112, first paragraph, by providing a reasonable written description for practicing the claimed invention. This is submitted to be the case even if the sequence as shown in Figure 1 of the specification is modified to the greatest extent suggested by the inventors and others (a change of no more than approximately 2%, at most).

The Board's attention is also drawn to the relative publication and filing dates of the art cited on paragraph spanning pp. 6-7 of the Office Action of May 24, 1991. The McDaniel et al. article was published in May 1988. Harper et al. was published in October 1988. The present patent application was filed in April 1989. Two subsequent references which discuss the sequence of the opd gene were published after the filing date of the present application and do not represent prior art. Mulbry et al. (cited by the Examiner in this rejection) was published

in December 1989, while Serdar et al. (cited in the Mulbry et al. reference as "in press") was published November 1989. Thus, while they are of interest for the reasons set forth below, the later two publications are not properly cited against the present application.

The Examiner correctly notes that there are differences between the sequences showing in McDaniel et al.. Harper, et al., Mulbry et al. and Figure 1 of the patent specification. The Examiner did not have the benefit of reviewing the non-prior art reference Serdar et al., but should he so have reviewed it he would have been differences between this reference and each (including Mulbry et al.) of the four sequences noted above, as well. However, Appellants submit that to characterize the sequences as only partially identical is a gross misstatement. The subsequently published sequences are, by any standard of comparison, overwhelmingly identical to the sequence for the opd gene originally discovered by the inventors.[2] In order to visualize how substantially identical these sequences are, the Appellants have produced a chart (Exhibit B), entitled "Comparison of opd Sequence Disclosed in Patent Application to Published Sequences." The Board's attention is directed to the appended Exhibit B for purposes of the remaining analysis of this basis for rejection.

There are a total of 1430 bases which overlap form all of the references noted above. The sequence originally obtained by the inventors and which was published in McDaniel et al. was corrected at nine (9) positions in the subsequent publication by the inventors (Harper et al.) which correlates to a percentage difference of less than 1.0% (0.63%). The inventors had, by the time that the patent application was to be filed, corrected the sequence by an additional small increment (including corrections of corrections made in the Harper et al. sequence). The difference between the Harper et al. sequence and that in Figure 1 of the present application is again only incremental (nine [9] base changes). Therefore, the best sequence known to the inventors at the time the application was filed is shown in the patent application at Figure 1 and only differs incrementally from the sequence originally published by the inventors.

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[2]This conclusion is acknowledged by Serdar et al. (1989), p. 1153, second column, third paragraph: "Most of the sequence was found to be identical with that obtained by this study."

35 U.S.C. § 112 mandates the disclosure of the best mode contemplated by the inventors. That the inventors were making every attempt to meet this requirement and in fact did os meet this requirement is evident in the disclosure of the sequence in Figure 1 which is an improvement over the sequence published in both McDaniel et al. and Harper et al.[3] This is made even more evident when comparing subsequently published sequences cited by the Examiner. Thus, by comparing the coding sequences between Figure 1 of the present application and the "corrected" sequence of Mulbry et al. (which was compared to the Harper et al. sequence only), one can readily see that twelve (12) of the alleged differences between the Harper et al. sequence and the Mulbry et al. sequence are, in fact, identical in the sequence disclosed in Figure 1 of the present application. The remaining differences between the Figure 1 coding sequence disclosed in the present application and that published by Mulbry et al. represent only approximately between 1.5%-2.1% (17/1144 or 26/1248 differences, respectfully) depending upon which termination signal is correct. Furthermore, even though the Mulbry et al. authors cite the in press article of Serdar et al. as disclosing an "identical" opd gene sequence, in fact there are several differences between these sequences as well.

The reason that this sequence has proven so difficult to accurately obtain is at least in part due to the relatively high G+C content of the DNA of the organisms from which this was isolated. Thus, there is a 53% (765/1430) G+C content in the PstI fragment sequenced by the inventors and others.[4] A phenomenon termed "CG compaction" causes considerable difficulty in many of the commonly available DNA sequencing techniques giving rise to spurious G's and C's. More recently available technology, such as that more recently employed by the inventors and others, has improved the ability of the DNA sequencer to overcome problems associated with GC compaction and similar anomalies.

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[3]It should be noted that:

> There is no statutory basis for reading into the best-mode portion a requirement that the mode disclosed be in fact the optimum mode for carrying out the invention. *In re Bosy*, 149 U.S.P.Q. 789 (C.C.P.A. 1966).

[4]The fact that this high G+C ratio contributes to the minor discrepancies in th publish d sequences is acknowledged by Serdar et al. (1989), p. 1154, column 1, last two lines.

The Board's attention is drawn to the fact that the vast majority of changes between the inventor's own investigations (McDaniel et al. and Harper et al.) and Figure 1 of the present application as well as those between Figure 1 of the present application and the subsequently published sequence of Mulbry et al. are, in fact, differences which add,delete or rearrange G's and C's in the sequence. Thus, for instance, of the differences between the sequence in Harper et al. of the present application and the analogous sequence from Mulbry et al. involve G's and C's. Likewise, virtually all of the differences between the sequence of Figure 1 and the sequences disclosed in the inventor's own references involve G's and C's.

It is Appellants' position that *Ex parte Marsili et al.*, 214 U.S.P.Q. 904 (PTO BD. App. 1979) is controlling in cases where originally disclosed chemical compounds require subsequent modification. It is also Appellants' position that *Ex parte Maizel*, to the extent it is distinguishable over the present case, is illustrative of the decisions and tests previously applied by the Board. These two cases will be discussed at length below in order to set the stage for Appellants' conclusion that the facts of the present case fall squarely under the *Ex parte Marsili et al.* decision and are distinguishable over those of *Ex parte Maizel*.

Ex parte Marsili et al.

In *Ex parte Marsili et al.*, an amendment to an originally-filed, complex chemical formula was necessitated when "[f]urther, more refined, analytical investigation showed" a minor modification was necessitated from that disclosed in the original patent application. A synthetic rifamycin (rifamycin-SV type), the compound discovered by the *Marsili* appellants, is a highly complex antibiotic which "looks" like a purine nucleoside 5'-triphosphate to bacterial RNA polymerase. Exhibit E. The chemical backbone of this compound has many substituents, one of which comprises an imidazoline ring. Initially, using less exacting methods, the appellants believed that there was a saturated bond in the ring. Later, using the better technology, they found that the ring, in fact, contained an unsaturated C-N linkage. This resulted not only in the addition of the double bond where none was thought to exist. It also resulted in the necessity to remove two hydrogens from the chemical structure and the molecular weight of the

compound. Relatively speaking, the loss of two hydrogen atoms resulted in an overall small change of the total numbers of atoms of a very large chemical compound comprising at least 40 additional hydrogen atoms, 37 carbon atoms, at least 2 nitrogen atoms, and 11 oxygen atoms (molecular weight with 2 additional hydrogen atoms = approximately 700; molecular weight without 2 additional hydrogen atoms = approximately 688; 0.3% change in molecular weight) (total numbers of atoms with 2 additional hydrogens = approximately 92; total numbers of atoms without 2 additional hydrogens = approximately 90; 2.2% change in total numbers of atoms).

The new matter rejection of the Examiner was reversed by the Board. The Board found that it was permissible to change the description of a novel isolated chemical since it was not necessary to add any characteristics not previously disclosed in the application. The Board found this to be distinct from those instances where an original description of a compound was insufficient. Relying on *In re Nathan*, 328 F.2d 1005 (Bd. Pat. App. 1964), the Board found that the where the requested change is an inherent characteristic of the claimed compound, that such a corrective change is not an addition of new matter to the specification.

> The Board stated it beliefs that:
>
> No one derives any benefit from an erroneous statement -- neither applicants nor the public.
>
> The *product*, not the formula or name, is the invention. Citing, *Petisi et al. v. Rennhard et al.*, 363 F.2d 903 (CCPA 1966).
>
> The PTO exists to carry out the job assigned it by Congress, pursuant to the Constitution (ARTICLE I, SECTION 8), i.e., to issue patents which "promote the Progress of Science and useful Arts." To refuse correction of the structural formula of Appellants' claimed compounds, . . . , would lead to the absurdity of issuing a patent which teaches the public in its specification the wrong scientific formula for the new products. *Marsili* at 906-907.

Ex parte Maizel

The First Hearing

In *Ex parte Maizel*, the question of correction of a DNA sequence originally disclosed, and later found to be incorrect was addressed. 27 U.S.P.Q.2d (PTO Bd. App. 1993). In that case, claim 1 (and similar claims) described a recombinant DNA vector which comprised any DNA sequence which encoded a protein of a defined range molecular weight, of a particular amino acid sequence, and having a BCGF biological activity. The claim language in describing the DNA sequence included the phrases "or a biologically functional equivalent thereof" or "corresponds biologically to." During prosecution, the appellants advised the Examiner that the DNA coding sequence set forth in the specification and drawings was in error. The appellants original sequencing of the DNA contained three (3) single base errors. Two (2) of the errors involved the necessity of deleting a single base at two different positions. These deletions caused the need to alter the predicted amino acid composition of the BCGF dramatically, and altered to some degree the predicted size of the protein. The examiner rejected claim 1 and similar claims on the ground that the subject matter that appellants wanted to claim was not described in the specification as filed, and such rejection was styled by the Board as a "new matter" rejection.

The Board recognized that errors may well arise in the sequencing of DNA and that a mechanism for correcting such errors in the Patent and Trademark Office is highly desirable. However, the Board declined to recommend a general rule, stating that the question of whether or not a change in the chemical structure of a DNA sequence set forth in the specification is permitted depends on the facts of each case and the significance of the modification to both the subject matter claimed (i.e., the invention), and the subject matter described in the specification.

The appellants in that case urged that the DNA sequence, although erroneous in the specification, was full described and enabled because a deposit of bacterial cells bearing the plasmid which contained the actual DNA sequence had been deposited with the American Type Culture Collection. It was also argued that *Ex parte Marsili* controlled and that the appellants should be allowed to correct the amino acid sequence of the recombinant BCGF because the claimed DNA was described adequately (either by the specification, or by the deposit, or by both, although it was not clear to the Board which was the basis of the appellants position).

However, the Board in that case declined to expand the *Ex parte Marsili* rationale to the appellants facts, holding *Ex parte Marsili* to its facts as follows:

> (1) *Marsili* involved claims to a complex chemical compound which were appealed because the appellant had discovered a minor error in the disclosed chemical structure after filing his application;
>
> (2) the appellant had provided analytical data and literature references to support the propriety and scientific desirability of the changes;
>
> (3) the original description of the compound in the specification included sufficient characteristics to distinguish the compound that was actually claimed; and,
>
> (4) there was no question of the threat of adding new characteristics not previously disclosed in the specification, being only a question of error in a structural formula.

The issue singled out by the Board for these types of cases is whether the description of the claimed compound in the original disclosure is adequate to identify and distinguish the claimed subject matter. *Citing, In re Nathan*, 328 F.2d 1005, 140 U.S.P.Q. 601 (C.C.P.A. 1964).

In distinguishing the facts of *In re Maizel*, the Board took the position that, unlike the facts of *In re Marsili*:

> (1) the appellants wished to make changes of a major nature and of the type recognized by the art as having potentially drastic consequences on the properties of the DNA and protein deduced therefrom;
>
> (2) appellants provided no evidence that the description of the invention set forth in the specification included sufficient characteristics to distinguish the invention set forth in the claims;
>
> (3) that there was little or no description of the DNA sequence of the claims other than the biological activity of the protein it codes for, its presence in the plasmids deposited in cells with the ATCC, and a rough estimate of the molecular weights of the fragments in those plasmids;
>
> (4) appellants did not purify and sequence the protein produced by the fragments on the plasmids as deposited (the plasmids encoded truncated proteins as opposed to the mature BCGF);
>
> (5) appellants described the protein only by its capability to be produced by bacterial cells

containing the plasmids and by its biological activity;

(6) appellants admitted that there were numerous molecules known to have BCGF activity;

(7) appellants claims do not describe the DNA directly but, rather, in terms of the protein and/or its amino acid sequence;

(8) appellants description of the protein solely in terms of its biological function and approximate molecular weight was insufficient to describe the protein and place appellants in possession thereof.

The Board concluded that at best the appellant's specification described the deposited cell line and the plasmid incorporated therein.

Further concluded the Board, the specification cannot be said to have adequately described the protein or the broad subject matter set forth in claim 1 (and others) because the amino acid sequence set forth as a descriptive parameter in the original specification was erroneously deduced and the protein was not purified and/or isolated. The Board concluded its affirmation of this basis of the rejection under § 112, first paragraph, stating, "In other words, the specification does not 'convey with reasonable clarity to those skilled in the art that, as of the filing date . . . [appellants were] . . . in possession *of the invention*,' i.e., '*whatever is now claimed.*' " *Citing, Vas-Cath, Inc. v. Mahurkar*, 953 F.2d 1555, 19 U.S.P.Q.2d 1111, 1117 (Fed.Cir. 1991).

The Reconsideration Hearing

The *Maizel* appellant requested reconsideration of the Board's decision, and in particular that portion of the decision in which the Board affirmed the examiner's rejection of claim 1 (and others) under § 112 as being directed to subject matter not described in the specification as filed, i.e., the "new matter" rejection. The Board summarized the appellant's arguments as: (1) the specification is enabling, especially when including the plasmid in bacterial cells in the ATCC deposit; (2) the specification inherently enables the sequence contained on the deposited plasmid; and, accordingly (3) satisfies the description requirement for the claimed subject matter.

The Board was again unpersuaded. The Board distinguished enablement and description as required under 35 U.S.C § 112. Quoting the Court of Appeals for the Federal Circuit, the Board agreed that:

> . . . we hereby reaffirm, that 35 U.S.C § 112, first paragraph, requires a 'written description of the invention' which is separate and distinct from the enablement requirement. The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed* (emphasis in *Maizel*; citations omitted in *Maizel*).

Next, the Board noted that the invention claimed was not the specific plasmid deposited but rather a generic invention directed to DNA which produces either a protein molecule of specified amino acid sequence, or a biological equivalent of that protein. The latter requirement in the claims was apparently key in the Board's decision to affirm the examiner's rejection.

The Board addressed the appellant's ATCC deposit. The Board determined that an ATCC deposit does not necessarily satisfy the description requirement of § 112. The Board analogized an ATCC deposit to that of a cross-reference to an earlier filed patent application for the preparation of a starting material. *Citing, Ex parte Schmidt-Kastner*, 153 U.S.P.Q. 473 (PTO Bd. App. 1963). Applying this analogy to the *Maizel* facts, the Board determined that the *Maizel* deposit did not satisfy the description requirement for either the DNA or the protein encoded by that DNA as they were claimed. The Board reached this result because there was no evidence on the record that the skilled artisan having the deposited material would have been aware of the BCGF DNA sequence or the BCGF sequence it encoded, nor would they have been able to accurately determine the DNA or amino acid sequences without undue experimentation. This argument was strengthened according to the Board because the appellant himself was unable to accurately sequence the DNA and protein until after the filing date of the application.

The Board determined that the DNA and protein sequences described in the specification were badly misdescribed. Because of this determination, the Board concluded that absent an

accurate DNA and protein sequences, the description of DNA remaining in the specification had to rely strictly on the molecular weight, biological activity and method of preparation (of the protein?), and that was insufficient under the facts of *Maizel*.

The Board cited the practical guidelines for determining when conception of an invention on biological subject matter occurs from *Amgen, Inc. v. Chugai Pharmaceutical Co.*:

> A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it . . . Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics distinguish it. *It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin*, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. (emphasis added in *Maizel*, 927 F.2d 1200, 1206, 18 U.S.P.Q.2d 1016, 1021 (Fed.Cir. 1991)).

Because, according to the appellant's specification, there were numerous biological molecules with the biological property of interest (BCGF-like activity), biological activity *per se* could not be said to be an adequate description of the DNA or protein claimed. Similarly, because there were many proteins of the same molecular weight as that described in the specification for the protein claimed, neither could molecular weight of the protein as disclosed in the specification be said to adequately describe the DNA or protein claimed. Absent these characteristics, the Board argued that only the method of making the plasmid remained as a mechanism for adequately describing the DNA.

Importantly, the *Maizel* specification did set forth a procedure for preparing the plasmids containing a cDNA capable of directing translation of an active protein. But, the Board pointed out that the plasmids deposited did not contain full-length inserts of the protein. It was even more critical to the Board's decision that out of the 700 clones which contained cDNA inserts, only the two resulting in the plasmids deposited coded for the active protein, and each of these was of a different molecular weight. The Board concluded that, therefore, even the method of

making the DNA as detailed in the specification failed to provide an adequate description, because there was no guarantee that either of the deposited plasmids would be reproduced by following the written description. Because an inaccurate DNA and protein sequences was described in the specification, and because the remaining mechanisms for describing the DNA could not rely on molecular weight, biological activity, or method of preparation of the DNA, the Board found the written description deficient.

The Board next addressed the scope of the *Maizel* appellant's claims *vis-a-vis* the scope of the specification description. The claims of *Maizel* were generic and were directed to recombinant vectors or cells comprising DNA sequences encoding a specific protein as set forth in the figures or "biologically functional equivalents thereof." Importantly, the *Maizel* claims did not describe the DNA by what it was ("an assemblage of nucleotides") but by what encodes (a protein). But, the deduced protein sequence was incorrect and misidentified the protein. Absent the correctly identified protein sequence, it is not possible to describe "biological equivalents thereof" even using conservative replacement theories of amino acid substitutions.

The Board took the position that there is substantial harm done the public when a "badly missequenced DNA" is subsequently allowed to issue in a patent, even if it is corrected prior to the issuance. This is because, according to the Board, that § 112 "new matter" rejections play an important role in establishing the filing dates of applications as *prima facie* evidence of invention (constructive, if not actual). *Citing, In re Hawkins*, 486 F.2d 569, 179 U.S.P.Q. 157 (C.C.P.A. 1973).

The Present Case

The case before the Board in this Appeal is similar to that in *Marsili* in almost every regard, the most notable exception being that a 1,300 base pair DNA fragment is a more complex chemical structure than a 37 carbon-backbone rifamycin molecule:

(1) Like *Marsili*, the present case involves claims to a complex chemical compound which are appealed because the present Appellants discovered minor errors in the disclosed highly complex and problematic chemical structure after filing the application;

(2) the Appellants have provided analytical data and literature references to support the propriety and scientific desirability of the requested changes;

(3) the original description of the compound in the specification includes sufficient characteristics to distinguish the compound that was actually claimed; and,

(4) there is no threat of adding new characteristics not previously disclosed in the specification, being only a question of error in a structural formula.

The present invention and the requested changes, on the other hand, are distinct from that of *Maizel* in many regards:

(1) the Appellants are not proposing changes of a major nature and of the type recognized by the art as having potentially drastic consequences on the properties of the DNA (no protein sequence is claimed);

(2) Appellants have provided ample evidence that the description of the invention set forth in the specification included sufficient characteristics to distinguish the invention set forth in the claims;

(3) there is ample description of the DNA sequence of the claims other than the biological activity of the protein it codes for, including not only its presence in a unique native plasmid deposited in cells with the ATCC but also it presence in an entirely unique compact DNA fragment of that plasmid the uniqueness of which cannot be denied, and a precise estimate of the molecular weights of the fragments in that plasmid;

(4) Appellants did purify and, at least to the extent possible, sequenced portions of the protein produced by the fragments on the plasmid as deposited (the plasmid encodes a complete, native protein);

(5) there is, to the extent known by the Appellants, only one molecule having such a organophosphorus acid anhydrase activity, and that is the molecule of the invention;

(7) Appellants claims directly describe the DNA sequence, and do not do so in terms of the protein and/or its amino acid sequence; and,

(8) the invention claimed is a specific method which utilizes a specific composition of matter, and is not a generic invention directed to DNA which produces either a protein molecule of specified amino acid sequence, or a biological equivalent of that protein.

The Claimed Invention

The present claims are method claims, not composition of matter claims, albeit claiming methods of using a composition comprising a specific DNA sequence. There is no protein sequence claimed (although a putative amino acid sequence is shown). There are no "biological equivalents" of the protein claimed.

Claims 53 and 61 are indicative of the methods claims, and in relevant part provide:

53. A method for detoxifying an organophosphorus compound comprising exposing said compound to recombinant bacterial organophosphorus acid anhydrase.

61. The method of claim 53 wherein said recombinant bacterial organophosphorus acid anhydrase is produced by a transformed microorganism comprising an expression vector for producing said anhydrase and wherein said vector has a cloned bacterial organophosphorus acid anhydrase gene fragment with the DNA coding sequence:

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5'
         CTGCAGCCTGACTCGGCACCAG . . .
ATG CAA ACG AGA AGG GTT GTG CTC . . .
met gln thr arg arg val val leu . . .

. . .  CAG GCA TCA CTG TGA
. . . gln ala ser leu  .  . . . 3'.
```

The Description of the DNA

The methods of the invention only refer to the DNA sequence of recombinant organophosphorus acid anhydrase, they do not require a specific amino acid sequence. Even if the DNA and deduced protein sequences are incorrect in some minor regard, the description of DNA remaining in the specification as to the size of the DNA fragment encoding the protein, the requisite and completely unique biological activity of the protein encoded by the DNA fragment, and precise foolproof method of preparing the DNA fragment, is sufficiently in contradistinction to the facts of *Ex parte Maizel* and sufficiently similar to the facts of *Ex parte Marsili* to merit the Board holding that a minor correction in the DNA sequence is allowable.

Errors in the DNA Sequence Are Minor

While it is vastly correct in its presently disclosed form, and while it is absolutely correct

(according to the best of the inventors' own results as well as the results of others) as to the critical 5' translational start site, the actual sequence outside of the critical 5' region is most likely something slightly different but between that disclosed in Figure 1 of the present application and the various sequences cited by the Examiner. In fact, this is precisely the current best understanding of the Appellants and results in a composite sequence believed by them to be the most correct sequence. It is submitted that the Appellants, in complying with the best mode requirements in the originally filed application, provided the sequence believed by them to be the most accurate. In only a handful of minor ways, the inventors have since modified their originally filed sequence. However, for this reason, the Appellants have proposed construction of a substitute Figure 1 which they request they be allowed to substitute for the originally field Figure 1. The proposed substitute would make only those conservative changes which are a consensus of the presently existing sequence, specifically that shown as the corrected sequence in Exhibit B.

In support of this substitute Figure 1 if allowed, a declaration of the inventors was proposed to be submitted as well stating that such substitute does not introduce any substantive new matter not the case since the corrected sequence is na inherent characteristic of the opd gene sequence enabled by the current invention. In so doing, Appellants submit that they will remain in full compliance with 35 U.S.C. § 112 first paragraph, by providing a reasonable written description for practicing the claimed invention. It is, therefore, requested that the Board allow the requested substitution informing Appellants' representative how best to achieve such substitution and remove this basis for rejection from the case.

Precise Characteristics of the DNA Fragment

The precise size of the DNA fragment is delimited by the PstI restriction sites on either side of the coding sequence. That fragment is precisely the 1.3 kb fragment of the native plasmid. That native plasmid is resident in the deposited strain and has but one 1.3 kb fragment. All of the other PstI fragments of that plasmid are either substantially larger than or substantially smaller than the 1.3 kb fragment encoding the opd gene.

The coding sequence for the gene itself practically subsumes the entire 1.3 kb fragment length. The start site is no more than about 60 bases from the 5' restriction site, and the stop site is little more than 200 bases from the 3' restriction site. As has been pointed out previously, there is no disagreement in the filed concerning the originally identified start site, the most critical aspect of the DNA sequence for purposes of hooking up the gene for expression and use in the methods of the invention presently appealed.

Unique Biological Activity of the Protein Encoded in the DNA

Likewise, there is but one enzyme known which fits the descriptions of the specification. As far as is known, even the enzymes from the disparate strains of bacteria have identical sequences and identical amino acid compositions, ergo identical activities. There are no biologically equivalent enzymes known, nor are any claimed.

Foolproof Method of Preparing the DNA

There is a foolproof way to obtain the precise DNA sequence necessary to carry out the claimed methods. That mechanism is carefully disclosed in substantial detail beginning with all the necessary starting materials and precise guidance at each step of the method in order to derive the exact DNA fragment, and therefore the exact DNA sequence necessary to carry out the hook-ups and recombinant enzyme expression necessary to obtain the recombinant enzyme used by the methods of the claims.

First, the specification provides at p. 7, l. 26-36--"The opd gene is isolated first by isolating the native plasmid DNA of ... Flavobacterium sp. (ATCC 27551)." A precise method for so doing is thereafter provided. Next, the specification provides at p. 23, l. 35 - p. 24, l. 13.--"The entire DNA from the degradative plasmid was digested with PstI (generating fragments of approximately 18.5, 17.3, 5.3, 4.3, 1.7, 1.6, 1.3, and 0.8 kb) and was subcloned into pBR322 within that vector's ampicillin gene. Cell free lysates of Ap^s clones selected from the Tc^r transformants of E. coli HB101-4442 [are then] tested for activity."

Unlike the methods of the *Maizel* inventors which relied on cDNA techniques and ran

the risk of allelic variations, there is no other result possible than the DNA sequence obtained by the present inventors, albeit slightly in error. In other words, the DNA sequence of the 1.3 kb fragment and the opd gene it encodes are inherent, much like the undisclosed double bond in the rifamycin-SV of *Marsili*. The present application provides a very straightforward, simple and foolproof way of obtaining the precise DNA fragment necessary to obtain the expression of the recombinant organophosphorus acid anhydrase of the claims. There is but a single result if the methods of the invention are used.

ISSUE V: Is the Disclosure Enabling Only for Claims Limited to The Specifically Disclosed Compounds Such as Parathion, Paraoxon, and Methyl Parathion?

NO. THE SPECIFICATION RECITES NUMEROUS OPs WHICH ARE SUSCEPTIBLE TO THE ENZYME, INCLUDING OP NERVE GASES

The Examiner has apparently overlooked evidence of numerous susceptible OPs in the specification. Page 32, line 16 *et seq.* illustrates this point admirably as shown there is a table showing that the enzyme was capable of degrading paraoxon, dursban, parathion, coumaphos, diazinon, fensulfothion, methyl parathion, and cyanophos. At page 33, lines 20 *et seq.*, there can be seen evidence of the enzyme degrading DFP, sarin, and soman. Moreover, the specification is quite clear concerning the substrate specificity of the recombinant enzyme. Page 31, line 20 *et seq.*, describes in some detail what types of OPs will be susceptible to the anhydrase attack. What makes this enzyme, in fact, so desirable to commercialize is the very breadth of range of substrates which it can attack. The specification cannot address each and every such substrate. However, it addresses such key substrates as have particular interest due to their toxicity or due to their prevalence of use.

In short, the specification teaches a large number of examples of substrates representing the genus of substrates susceptible to the cleavage by the anhydrase, and teaches generically what sorts of OPs will be substrates. Appellants know of no way short of listing each and every one of the numerous such substrates of this enzyme to claim the class of substrates other than the manner in which it has been done in the claims on appeal. Appellants maintain that the

breadth of the claims, when read as is proper in conjunction with the specification, is accurate.

(ii) Rejections Under 35 U.S.C. § 112, Second Paragraph

ISSUE VI: Are the Claims Indefinite for Failing to Particularly Point Out and Distinctly Claim the Subject Matter Which Applicant Regards as the Invention?

NO. THE CLAIMS RECITE: (1) "EXPOSING" AN OP TO THE ENZYME SINCE A VARIETY OF WAYS OF ACHIEVING THE REACTION MAY BE ACCOMPLISHED; AND, (2) "ORGANOPHOSPHORUS COMPOUND" IS THE BEST KNOWN CHEMICAL TERM TO DESCRIBE THE WIDE RANGE OF OPs SUSCEPTIBLE TO DEGRADATION BY THE ENZYME.

The Examiner has objected to the use of "exposing" in reference to the method of detoxifying the OPs using the recombinant enzyme. Appellants utilize this term in order to connote the numerous ways in which the specification discloses such detoxifications to occur. Other words such as "contacting," "interacting," "reacting," etc. would be equally but no more descriptive of these numerous processes. Certain other words such as "mixing," "dissolving," "solvating," etc. would be poor choices since these terms may connote a solution or liquid matrix which would not account for the instance when air-borne OPs are brought into contact with the recombinant enzyme resulting in their detoxification.

Likewise, the Examiner has objected to the term organophosphorus. Exhibits F-K indicate that the Environmental Protection Agency refers to the compounds in a manner consistent with that used by the Appellants. These *Pesticide Fact Sheets* each indicate that the chemical family by which the specific OP compounds shown to be susceptible to the recombinant enzyme of the invention are "organophosphates." While it is true that there are molecules which contain both carbon (C: "organo") and phosphorus (P; "phosphorus," "phosphate") that are not substrates of the enzyme, the best known term to inclusively cover the broad range of compounds which are substrates of the enzyme is "organophosphorus."

(iii) Rejections Under 35 U.S.C. § 102

ISSUE VII: Are Claims 53, 54, and 58-63 Anticipated by McDaniel *et al.*?

NO. MCDANIEL ET AL. IS NOT PRIOR ART.

A. The Examiner's Rejection

The Examiner has rejected certain of the claims under 35 U.S.C. § 102(a) as being anticipated by McDaniel et al. (BY). The Examiner takes the position that McDaniel et al. discloses cloning and expression of an opd gene encoding a phosphotriesterase using the same strains, vectors, restriction enzymes and DNA fragment. The Examiner further notes the unexplained disparity of the sequences noted previously and concludes that the DNA sequences are the same.

The Examiner also takes the position that this same reference forms the basis for a rejection under 35 U.S.C. § 103. This rejection will be treated separately below.

B. Appellants' Remarks

The Board's attention is again drawn to the relative publication and filing dates of the art cited. The McDaniel et al. article was published in May 1988. The present patent application was filed in April 1989.

MPEP 715.01(c) states:

> Where the applicant is one of the co-authors of a publication cited against his application, he is not required to file an affidavit or declaration under 37 C.F.R. 1.131. The publication may be removed as a reference by filing a disclaiming affidavit or declaration of the other authors. *Ex parte Hirschler*, 110 U.S.P.Q. 384.

However, a co-author's disclaiming affidavit is apparently not necessarily required. In *In re Katz*, 687 F.2d 450,215 U.S.P.Q. 14 (CCPA 1982), the Court held that disclaiming affidavits were not necessary. The Court clearly stated that authorship does not give rise to any presumption regarding inventorship. The only requirement appears to be a "reasonable showing supporting the basis for the applicant's position." 215 U.S.P.Q. at 18. The Examiner, under this case law, is not free to speculate about the alternatives in the face of the applicant's own sworn satisfactory explanation. *See, In re Kusko*, 215 U.S.P.Q. 972 (PTO Bd. App. 1981) (reaching the same opinion).

The Declaration of Invention filed in conjunction with the present application is submitted by Appellants to be in accord with the present application is submitted by Appellants to be in accord with the relevant case law cited above in providing a "sworn satisfactory explanation" of the inventorship of the present application.[5] The reference McDaniel et al. was co-authored by McDaniel, Harper and Wild. The reference Harper et al. was co-authored by Harper, McDaniel, Miller and Wild. Co-inventor Raushel was not an author on either of these articles. The additional co-authors of both the McDaniel et al. and Harper et al. references were either technicians (Harper) or students (Miller) working in conjunction with the inventors in order to reduce the inventions of McDaniel, Wild and Raushel to practice.

It is believed by the Appellants that this further explanation in combination with the originally filed sworn Declaration of Inventorship is sufficient to fully overcome the rejection under 35 U.S.C. § 102(a) cited against the application in view of cases such as *In re Katz*. The principal inventor who directed the work of both Harper and Miller (McDaniel), is in fact the prosecuting attorney of the case and is the person who signed the statements regarding his supervision of Harper and Miller in merely reducing to practice his and the other inventors inventions. He was, as were all of the other inventors (and, for that matter, Harper and Miller) under a common duty to assignee any and all such inventions to the common original assignee (Texas A&M University). It is, therefore, maintained by the Appellants that mr. Mcdaniel's statement and signature as an inventor and as the prosecuting attorney moots the requirement for a separate declaration. However, should the Board so require, a disclaiming affidavit from both Harper and Miller will be obtained and submitted.

ISSUE IX: Are Claims 53, 54, and 58-63 Anticipated By Harper *et al.*?

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[5]The relevant portion of the Declaration sworn to by each of the three inventors of the present application and filed therewith in the Patent and Trademark Office states:

> I believe I am the original, first and sole inventor (if only one name is listed below) or the below named inventors are the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled RECOMBINANT ORGANOPHOSPHORUS ACID ANHYDRASE AND METHODS OF USE the sp cification of which is attached hereto.

NO. HARPER ET AL. IS NOT PRIOR ART.

A. The Examiner's Rejection

The Examiner has rejected certain claims under 35 U.S.C. § 102(a) as being anticipated by Harper et al. (BY). Similarly to the rejection stated for McDaniel et al. above, the Examiner takes the position that McDaniel et al. discloses cloning and expression of an opd gene encoding a phosphotriesterase where the DNA sequence is the same for p. diminuta and a Flavobacterium sp. (ATCC 27551). The Examiner again notes that the same strains, vectors, restriction enzymes and DNA fragment disclosed in the reference are used in the patent application. The Examiner again further notes the unexampled disparity of the sequences noted previously and concludes that the DNA sequences are the same.

The Examiner also takes the position that this same reference forms the basis for a rejection under 35 U.S.C. § 103. This rejection will be treated separately below.

B. Appellants' Remarks

The Board's attention is again drawn to the relative publication and filing dates of the art cited. Harper et al. was published in October 1988. The present patent application was filed in April 1989.

As noted above, the same arguments apply as an explanation of the authorship and inventorship of the McDaniel et al. and Harper et la. publications. It is believed by the Appellants, as argued above, that this statement is sufficient to fully overcome the rejection under 35 U.S.C. § 102(a) cited against the application in view of cases such as *In re Katz*. However, should the Board so require, a disclaiming affidavit from both Harper and Miller will be obtained and submitted.

ISSUE XI: Are Claims 53, 58, and 60 Anticipated By Wild *et al.*?

NO. WILD ET AL. TEACHES AWAY FROM THE PRESENT INVENTION. ITS TEACHINGS WOULD UTTERLY FAIL TO PROVIDE THE NECESSARY DNA AND RECOMBINANT PROTEIN.

The Examiner has rejected certain claims under 35 U.S.C. § 102(b) as being anticipated by Wild et al. (AT) or Mulbry et al. (AY). The Examiner takes the position that Wild et al. discloses cloning and expression of organophosphorus degrading genes from P. diminuta and a Flavobacterium and that Mulbry et al. discloses cloning and expression of organophosphorus genes from P. diminuta and a Flavobacterium (ATCC 27551)using a cloned DNA fragment that contained the opd gene derived from P. diminuta. The Examiner additionally takes the position that while the DNA sequence is not disclosed in either of the references, that only "routine sequencing would have been needed to determine the sequence."

The Examiner also takes the position that this same reference forms the basis for a rejection under 35 U.S.C. § 103. This rejection will be treated separately below.

B. Appellants' Remarks

Wild et al. does not teach the DNA sequence of the opd gene nor does it anticipate the difficulty that the present inventors encountered in obtaining the sequence and the initiation codon necessary for subsequent manipulation of the opd gene for purposes of the critical expression of the gene.

The Board's attention is drawn to the fact that the Wild reference does not teach where the open reading frame occurs within the originally isolated fragment from the soil bacteria. In fact, the Wild reference teaches that it is possible to enhance expression of the opd gene product by removing approximately 250 base pairs of DNA from the 5' flanking sequence of the fragment. To the contrary, the present invention teaches that removal of such a region of DNA from the opd gene-containing fragment eliminates any OPA activity (see, Fig. 2). Thus, if one were to follow the teachings of the Wild reference, one would place the initiation site of the opd gene at least 250 base pairs down stream of the PstI site and approximately at least 190 base pairs away from the actual initiation site. In fact, were one to make such a construction by deleting the first 250 base pairs from the PstI fragment one would throw away the fragment containing the actual initiation site. What the disclosures of the Wild reference reiterates, in fact, is the confused state of the art prior to the present invention.

Thus, while there are some similarities between the elements of the present invention and the Wild reference, the critical discoveries which allowed the inventors and others to successfully clone, sequence, and express high levels of opd gene product had to await the inventions described in the present application. For these reasons, the Appellants submit that the Wild reference is improperly cite by the Examiner as a barring reference under 102(a). It is, therefore, requested that this basis of rejection be removed from the case.

ISSUE XIII: Are Claims 53, 54, and 60 Anticipated By McDaniel?

NO. MCDANIEL TEACHES AWAY IN IMPORTANT REGARDS.

A. **The Examiner's Rejection**

The Examiner has rejected certain claims under 35 U.S.C. § 102(b) as being anticipated by McDaniel (AZ). The Examiner takes the position that the McDaniel reference discloses cloning the expression of an opd gene encoding a phosphotriesterase using the same strains, vectors, restriction enzymes and DNA fragment as the present invention disclosure. The Examiner in particular notes at least page iii, the tables, page 46, 55-56, 69, figures 17 and 19, pages 82, 89-91 and 116-120. The Examiner correctly points out that although sequencing was performed, there was no disclosure of the sequence in the reference.

The Examiner also takes the position that this same reference forms the basis for a rejection under 35 U.S.C. § 103. This rejection will be treated separately below.

B. **Appellants' Remarks**

The McDaniel reference (AZ) represents a Ph.D. dissertation of one of the inventors. It was preliminarily in nature as to the studies which were ultimately to give rise to the inventions of the present application. Clear evidence of the preliminary nature of this reference is the fact that the DNA sequence of the opd gene is not disclosed herein although attempts to obtain such a sequence were clearly carried out as shown in the reference at the points recognized by the Examiner. The inventors were unable at the time of the publication of this reference to even provide preliminary sequencing information since the difficulties heretofore described prevented them from accurate sequencing.

Thus, while the McDaniel reference does give an indication of research in progress which led to the invention, it clearly does not teach one of skill in the art how to make the invention. In fact, the McDaniel reference teaches away from the current invention in important ways. For these reasons, Appellants submit that the McDaniel reference does not anticipate the present invention and should, therefore, be removed as a basis for rejection of the claims.

ISSUE XIV: Are Claims 61-63 Anticipated By McDaniel?

NO. MCDANIEL TEACHES AWAY IN IMPORTANT REGARDS.

Again, clear evidence of the preliminary nature of this reference is the fact that the DNA sequence of the opd gene is not disclosed herein although attempts to obtain such a sequence were clearly carried out as shown in the reference at the points recognized by the Examiner. The inventors were unable at the time of the publication of this reference to even provide preliminary sequencing information since the difficulties heretofore described prevented them from accurate sequencing. The rejected claims each specifically require the recombinant DNA sequence of the invention.

Thus, while the McDaniel reference does give an indication of research in progress which led to the invention, it clearly does not teach one of skill in the art how to make the invention. In fact, the McDaniel reference teaches away from the current invention in important ways. For these reasons, Appellants submit that the McDaniel reference does not anticipate the present invention and should, therefore, be removed as a basis for rejection of the claims.

(iv) Rejections Under 35 U.S.C. § 103

ISSUE VIII: Are Claims 53, 54, 58, and 59-63 Obvious Over McDaniel *et al.*?

NO. MCDANIEL ET AL. IS NOT PRIOR ART, PURSUANT TO THE ARGUMENTS RAISED ABOVE.

ISSUE X: Are Claims 53, 54, 58, and 59-63 Obvious Over Harper *et al.*?

NO. HARPER ET AL. IS NOT PRIOR ART, PURSUANT TO THE ARGUMENTS RAISED ABOVE.

ISSUE XII: Are Claims 61-63 Obvious Over Wild *et al.*?

NO. WILD ET AL. TEACHES AWAY FROM THE PRESENT INVENTION. ITS TEACHINGS WOULD UTTERLY FAIL TO PROVIDE THE NECESSARY DNA AND RECOMBINANT PROTEIN, PURSUANT TO THE ARGUMENTS RAISED ABOVE.

ISSUE XV: Are Claims 61-63 Obvious Over McDaniel?

NO. MCDANIEL TEACHES AWAY IN IMPORTANT REGARDS, PURSUANT TO THE ARGUMENTS RAISED ABOVE.

ISSUE XVI: Are Claims 53-54 and 59-64 Obvious Over Munnecke I, Taken With Munnecke II, McDaniel *et al.*, and Gottlieb?

NO. MUNNECKE I IS A GENERAL REFERENCE WHICH MERELY ALLUDES TO THE DESIRABILITY OF TREATING PESTICIDES WITH MICROORGANISMS, CRUDE NATIVE ENZYMES AND PURIFIED, NOT RECOMBINANT PESTICIDES; MUNNECKE II MERELY SHOWS THE UTILITY OF USING A CRUDE OP DEGRADING ENZYME FROM A MIXED BACTERIAL CULTURE; MCDANIEL ET AL. IS NOT PRIOR ART; GOTTLIEB LIKE MUNNECKE I AND II IS A REFERENCE WHICH ALLUDES TO THE USE OF NON-RECOMBINANT ENZYMES IN DEGRADATION OF GASEOUS OPs; MCDANIEL ET AL. IS NOT PRIOR ART.

Munnecke I does not refer to any recombinant enzyme. It does not refer to any OP-degrading OP enzyme. The reference is, in fact, merely a very general reference which discusses the desirability of using crude extracts of microbial enzymes to detoxify pesticides. Munnecke II also fails to recite any teaching of a recombinant enzyme useful in OP degradation. This reference merely teaches that a crude OP-degrading activity derived from a mixed bacterial culture of unknown constituency may be used to degrade OPs when immobilized in a column of resin. Gottlieb, like Munnecke II, merely alludes to the use of immobilized crude enzymes, none of which are recombinant, to treat gaseous phase OPs. McDaniel et al. is not prior art, for the reasons stated above.

ISSUE XVII: Are Claims 53-54 and 59-64 Obvious Over Munnecke I Taken With

Munnecke II, Wild *et al.*, and Gottlieb?

MUNNECKE I, MUNNECKE II, AND GOTTLIEB ARE DISTINCT AND DO NOT TEACH RECOMBINANT ENZYMES AS ARGUED ABOVE; WILD ET AL. TEACHES AWAY FROM THE PRESENT RECOMBINANT ENZYME FOR THE REASONS STATED ABOVE

ISSUE XVIII: Are Claims 55-57 Obvious Over Munnecke I Taken With Munnecke II, McDaniel *et al.*, and Gottlieb, or Obvious Over Munnecke I Taken With Munnecke II, Wild *et al.*, and Gottlieb As Applied To Claims 53-54 and 59-64, and Further In View of Grot *et al.*?

MUNNECKE I, MUNNECKE II, AND GOTTLIEB ARE DISTINCT AND DO NOT TEACH RECOMBINANT ENZYMES AS ARGUED ABOVE; WILD ET AL. TEACHES AWAY FROM THE PRESENT RECOMBINANT ENZYME FOR THE REASONS STATED ABOVE; MCDANIEL ET AL. IS NOT PRIOR ART; GROT IS A NON-ENZYMATIC METHOD OF PROTECTING AGAINST OP TOXICITY

Each of Munnecke I, Munnecke II, Gottlieb, and Wild et al. are distinct and do not teach the invention alone or in combination for the reasons stated above. McDaniel et al. is not prior art for the reasons stated above.

Grot is a reference which incorporates a highly fluorinated ion exchange polymer bearing a sulfonic acid functional group into a protective garment for the purposes of degrading OPs. There is no teaching of an enzymatic detoxifying enzyme. Moreover, there is no teaching of a recombinant OP-degrading enzyme. Grot is not, therefore, a reference properly combinable or one which if viewed alone, teaches the methods of using a recombinant OP-degrading enzyme as that of the invention to detoxify OPs.

Appellants' General Remarks

It is respectfully submitted that the foregoing references, alone or in combination, fail to teach or even to suggest any of Appellants' invention, but in particular fail to teach or even to suggest the steps necessary to accurately sequence the opd gene, to obtain the necessary information to determine the start site of the gene, which knowledge is absolutely necessary in

order to modify the DNA in a manner to allow commercial scale heterologous expression of the OPA enzyme and which knowledge is absolutely necessary to obtain heterologous expression of the membrane-associated enzyme from soil bacteria in eukaryotic systems. The proper context for determining the issue of obviousness or nonobviousness is the Supreme Court's decision in *Graham v. John Deere*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966) that sets forth the following considerations:

(1) The scope of and content of the prior art;

(2) The differences between the prior art and the claims at issue;

(3) The level of ordinary skill the pertinent art; and

(4) Secondary considerations such as commercial success, long-felt and unresolved needs, failures of others, etc.

See MPEP §§ 706.

1. **Scope and Content of the Prior Art**

a. **McDaniel et al. Does Not Qualify As 35 U.S.C. § 103 Prior Art**

The Board's attention is again drawn to the Appellants' arguments above relating to removal of this reference as a publication bar to the patentability of the present application. It is believed by the Appellants that the arguments above are similarly sufficient to fully overcome the rejection under 35 U.S.C. § 103 cited against the application in view of cases such as *In re Katz*. However, Appellants wish to reiterate that should the Board so require, a disclaiming affidavit from both Harper and Miller will be obtained and submitted.

b. **Harper et al. Does Not Qualify as 35 U.S.C. § 103 Prior Art**

The Board's attention is drawn to the arguments addressed in "a." above. It is believed by the Appellants, as argued above, that these arguments are sufficient to fully overcome the rejection under 35 U.S.C. § 103 cited against the application in view of cases such as *In re Katz*.

However, should the Board so require, a disclaiming affidavit from both Harper and Miller will be obtained and submitted.

c. **Wild et al.: (1) does not teach the DNA sequence of the opd gene; (2) does not anticipate the difficulty that the present inventors encountered in obtaining the sequence and the initiation codon necessary for subsequent manipulation of the opd gene for purposes of the critical expression of the gene; (3) does not teach means for overcoming any of the difficulties encountered by the Applicants; and (4) does clearly teach away from the present invention. Moreover, there is nothing in Wild et al. which suggest the combinations proposed by the Examiner.**

As was pointed out previously, there are at least four published versions of the opd gene sequence, three of which were carried out by different laboratories using different techniques, yet presumably of the identical DNA sequence. Moreover, the sequence disclose din the present patent application is yet another version of the same sequence derived with substantial research and development by the present inventors. While all of these sequences are substantially the same, the fact that there sis not even to date a fully agreed upon sequence between those involved in the research in this field clearly points out the difficulty encountered by the inventors in obtaining a sequence for the gene they had isolated.

More importantly for the purposes of the present invention however, is the difficulty that was encountered in obtaining the translational open reading frame for the opd gene. Unlike genes isolated from the bacterium Escherichia coli, the genes isolated from soil bacteria like those of the present invention were not well-characterized at the time of the making of the present invention. Only a handful of Pseudomonas genes had been isolated at the time of the present invention and no Flavobacterium genes had been isolated to the inventors' knowledge.

Thus, while it was reasonable to presume that the initiation codon would be an ATG codon, it was just as reasonable to assume that the initiation codon was GTG (at lest some art of which the inventors were aware at the time of the making of the invention indicated GTG was a possible initiation codon in bacteria) and, in fact may have been an initiation codon altogether novel unlike the classical enteric bacteria. This increased the potential starting sites for the gene substantially especially int eh GC-rich DNA of these soil bacteria. The fact that the initiation codon most likely included at lest one, and possibly tow, G nucleotide(s) further complicated the search for the proper coding sequence of the opd gene. This was particularly true since the inventors knew that they were likely to be at least partially incorrect as to the actual G's and C's in the sequence due to the phenomenon of GC compaction discussed above.

Further difficulty was quickly encountered when the inventors tried to sequence the minuscule amounts of partially purified organophosphorus acid anhydrase (OPA) obtained from E. coli cells transformed with the heterologous opd gene having failed to do so adequately in the sot organisms. The very low activities associated with normal expression techniques as illustrated in the specification at p. 9 are indicative of the problems encountered by the inventors and others in obtaining enough purified protein to sequence for purposes of determining the actual coding sequence for the opd gene. Thus, as pointed out in the specification at p. 23, lines 10-34 and p. 25, lines 8-13, it became necessary for the inventors to use fusion proteins in order to overcome the substantial roadblocks they and others had encountered in purifying the sequencing the OPA enzyme. Moreover, in order to achieve the substantial purifications of the invention, effective amounts of expression of the membrane-associated enzyme had to be

achieved in a heterologous cell such as E. coli or in baculovirus-infected insect cells.

The Board's attention is additionally drawn to the fact that the Wild reference, which was s preliminary report in the earliest stages of the inventors' research, does not teach where the open reading frame occurs within the originally isolated fragment from the soil bacteria. In fact, the Wild reference teaches that it is possible to enhance expression of the opd gene product by removing approximately 250 base pairs of DNA from the 5' flanking sequence of the fragment. To the contrary, the present invention teaches that removal of such a region of DNA from the opd gene-containing fragment eliminates any OPA activity (See, Fig. 2). Thus, if one were to follow the teachings of the Wild reference, one would place the initiation site of the opd gene at least 250 base pairs down stream of the PstI site and approximately at least 190 base pairs away from the actual initiation site. In fact, were one to make such a construction by deleting the first 250 base pairs from the PstI fragment one would throw away the fragment containing the actual initiation site. What the disclosures of the Wild reference reiterates, in fact, its the confused state of the art prior to the present invention

As noted previously, these type problems were recently the focus of concern for the CAFC. In *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200 (Fed. Cir. 1991), the defendants asserted error in the district court's legal conclusion that in this case the inventor's conception occurred simultaneously with reduction to practice. The codefendants claim the inventor was the first to conceive a probing strategy of using two sets of fully-degenerate cDNA probes of two different regions of the EPO gene to screen a gDNA library.

Defendants here further claimed that another inventor conceived this strategy in 1981, was diligent until he reduced it to practice in May of 1984 and thus should be held to be a § 102(g) prior art inventor over Amgen's inventor who reduced the invention to practice in September 1983.

The CAFC was presented, therefore, with the question of when exactly, in the cloning and sequencing of a previously unknown DNA sequence whose encoded amino acid sequence is also unknown, does reduction to practice occur. The Court held:

> Prior to 1983, the amino acid sequence for EPO was uncertain, and in some position the sequence envisioned was incorrect. Thus, until Fritsch (Chugai's inventor) had a complete mental conception of a purified and isolated DNA sequence encoding EPO and a method for its preparation, in which the precise identity of the sequence is envisioned, or in terms of other characteristics sufficient to distinguish it form other genes, all he had was an objective to make an invention which he could not then adequately describe or define.

It is submitted by Appellants here that a similar situation existed in the invention of the present application. The amino acid sequence for OPA was entirely unknown prior to the disclosure by Appellants in a journal article less than one year prior to the filing of the present invention. Until that item, the location of the gene, the correct gene sequence, the start site and the putative amino acid sequence were not only purely speculative but actually incorrectly characterized. Thus, until the present inventors had "a complete mental conception of a purified and isolated DNA sequence" encoding the correct OPA amino acid sequence, "and a method for its preparation, in which the precise identity of the sequence is envisioned, or in terms of other

characteristics sufficient to distinguish it from other genes," all the present inventors or any others had was "an objective to make an invention" which could not then be adequately described or defined.

Thus, while there are some similarities between the elements of the present invention and the Wild reference, the critical discoveries which allowed the inventors and others to successfully clone, sequence, and express high levels of opd gene product had to await the inventions described in the present application. For these reasons, the Appellants submit that the Wild reference is improperly cited by the Examiner as a barring reference under § 103. It is therefore, requested that this basis of rejection be removed from the case.

e. **The McDaniel (AZ) reference does not disclose the opd gene sequence and, moreover, teaches away from the present invention in a number of critical ways.**

The McDaniel reference (AZ) represents a Ph.D. dissertation of one of the inventors. It was preliminary in nature as to the studies which were ultimately to give rise to the inventions of the present application. Clear evidence of the preliminary nature of this reference is the fact that the DNA sequence of the opd gene is not disclosed herein although attempts to obtain such a sequence were clearly carried out as shown in the reference at the points recognized by the Examiner. The inventors were unable at the time of the publication of this reference to even provide preliminary sequencing information since the difficulties heretofore described prevented them from accurate sequencing.

Moreover, even the limited sequencing that was carried out resulted in a serious error

in estimating the initiation site of the opd gene. This is clearly indicated in Table 9 on page 100 of this reference. Here it can be seen that the opd gene is said to possess a GTG start site and various promoter sequences 5' thereof. In actuality, the bona fide start site is some 18 base pairs from the GTG identified in this reference and the 5' promoter regions are vastly different than those shown in the Table 9 of this reference (it is to be noted that the largest part of these differences are accounted for by the GC compaction problem noted before). Thus, if the skilled technician sued the teachings of the McDaniel reference, he would clearly mistake the actual start site of the opd gene since it teaches away from the ATG site identified through substantial effort and inventive approaches to sequencing the DNA and protein as taught by the present patent application.

Furthermore, the McDaniel reference clearly points out this failure of the inventors at the time of the publication of this reference to determine the actual start site of the gene. The Board's attention is drawn to p. 98, liens 21-24 and p. 101, lines 1-2 of the McDaniel reference. It is made clear here that clarification of the start sites of the opd gene would require S1-nuclease mapping and/or purification and sequencing of the gene product. It was also made clear that these techniques were unavailable to the inventors as tools since purification of the membranae-bound enzyme and the isolation of mRNA had not been feasible. Had, of course, the DNA sequence on the start site and open reading frame been available to the inventors at the time of publishing this reference, it would most certainly have been included in its entirety and fully characterized. It was not since data were preliminary, flawed and internally inconsistent at the time.

The Board's attention is drawn to the important fact that there is no teaching the McDaniel reference which definitively shows a molecular weight of the OPA enzyme. Very clearly, molecular weight gels were inconclusive as to the weight of the protein (see Fig. 22). The approximately 1300 bp fragment isolated in this study potentially could code for a protein ranging from 10,000 to 65,000 Da ([1300 bp ÷ 3 bp/amino acid] x ~150 ^{Da}/amino acid ≡ 65,000 Da). There was no way to determine where, in this range, the OPA fell. The inventors at the time of the drafting of this reference postulated the existence of a 31,000 Da protein (as shown in Fig. 22 of the reference). However, this could not be confirmed since there were other prominent bands ranging from the 65,000 range and down (*see e.g.*, band at approximately 26,000 Da) with similar characteristics and representing as likely a candidate protein.

Thus, while the McDaniel reference does give an indication of research in progress which led to the invention, it clearly does not teach one of skill in the art how to make the invention. In fact, the McDaniel reference teaches away from the current invention in important ways. For these reasons, Appellants submit that the McDaniel reference does not anticipate the present invention. Nor does this reference teach or suggest the combinations proposed by the Examiner and should, therefore, be removed as a basis for rejection of the claims.

The Munnecke I, Munnecke II, Gottlieb, and Grot references do not discuss methods of using recombinant enzymes and will not be further addressed here. Instead, Appellants refer to their arguments above as to each of these references.

2. The Differences Between the Claims and the Cited Art

Claim 53 and those depending from it relates to a method of using a recombinant bacterial organophosphorus acid anhydrase

No reference nor combination of references proposed by the Examiner teaches or suggests the requisite inventions of both a substantially purified and isolated bacterial organophosphorus acid anhydrase gene and the DNA sequence coding for the enzyme. Only with the teaching of the present disclosure is it possible to locate the start signal and open reading frame of the opd gene. Without these teachings, one is left with little more than has been known in the art for years. Without the sequence of the gene disclosed in the present invention, and in particular with the inability of the prior art to isolate and purity the OPA enzyme, no prior art references alone or in combination teach the skilled artisan the manner of locating, isolating or purifying the actual opd gene.

Claim 53 and those depending from it relates a recombinant enzyme capable of degrading OPs, which recombinant enzyme is encoded in an integral opd gene with known start signal and defined sequence, contained within a small DNA fragment derived from a native plasmid.

No reference nor combination of references teaches or suggest the requisite knowledge of the start site and the characterized sequence necessary to produce OPA from a recombinant vector as claimed. Only by following the teachings of the present invention could one of skill in the art have predictably and with relative certainty obtained such requisite information. The Board's attention is again drawn to the limitations that existed at the time of the making of the present invention.

(1) Few sequences exited for genes from soil bacteria, in particular, no sequences were known for Pseudomonas diminuta or Flavobacterium bacterial genes;

(2) Thus, there was no way to predict for certain the nature of the start site, promoter, internal sequences, terminators and the like for these soil bacteria;

(3) The DNA of these bacteria was known to have a high GC content and, thus, the sequencing was commensurately difficult;

(4) The difficult in sequencing (GC compaction) caused the inventors numerous difficulties since the likelihood that G's and C's were either missing or wrongly placed substantially increased the likelihood of incorrect placement of start signals containing G's ("ATG" or "GTG" were the signals known at the time of the making of the invention)p;

(5) Certain of the prior art references clearly taught away from the present invention;

(6) The prior art references must lead one of skill in the art to make the combinations of art, which none of the references do; and,

(7) Even if the prior art references are properly combinable, they do not teach the necessary elements of the start site and the gene sequence.

Claim 53 and those depending from it relate to the expression of opd in microorganisms or eukaryotic cell lines (i.e. "recombinant"). There is simply no teaching or suggestion in the art that indicates heterologous expression would be possible at levels necessary to achieve the successful purification of the membrane-associated protein OPA.

The Board's attention is additional drawn to the failure of any prior art reference to teach or suggest the combination of the opd gene with a heterologous promoter for heterologous expression in a host other than the original host. This failure in the prior art is a direct result of the lack of knowledge about the start site and sequence provided by the present invention. Even where the opd gene is expressed under the control of its own promoter, the increased expression is achieved in a soil bacterium and occurs by control of the copy number of the vector.

Moreover, there is nothing in the art that suggests that a membranae-associated protein

from soil bacteria could be expressed as an active protein in an eukaryotic cell line.

Therefore, it is respectfully submitted that no reference alone or in combination teach the critical aspects of the present invention. Even if the references are properly combinable as proposed by the Examiner, the combinations do not derive the teachings disclosed in the present invention.

3. **Level of ordinary Skill in the Art**

The *Graham* inquiries point to a conclusion of nonobviousness of the present claims regardless of the presumed level of ordinary skill in the art. However, absent evidence to the contrary, a person or ordinary skill in the art is presumed to be one who essentially follows conventional wisdom and does not undertake to innovate. As stated by the Federal Circuit in *Standard Oil Co. v. American Cyanamid Co.*, 227 U.S.P.Q. 293, 298 (Fed. Cir. 1985):

> A person of ordinary skill in the art is also presumed to be one who thinks along the line of conventional wisdom in the art and is not one who undertakes to innovate, whether by patient, and often expensive, systematic research or by extraordinary insights, it makes no difference which. (Emphasis supplied)

Appellant submits that one who follows conventional wisdom would not extrapolate the subject matter of the present claims from the teachings of the references proposed by the examiner. Accordingly, it is submitted that none of the references are combinable in the manner supposed by the Examiner. More importantly, there is clearly no motivation in any of these references relied upon by the Examiner to make the combinations proposed by the Examiner.

Specifically, none of the cited references suggest. The requisite start signal and gene sequence disclosed only in the present application. As the Patent and Trademark Office Board of Appeals stated in the case of *Ex Parte Chicago Rawhide Manufacturing Co.*, 223 U.S.P.Q. 351, 353 (PTO Bd. App. 1984);

> The prior art must provide a motivation or reason for the worker in art, without the benefit of the Appellant's application, to make the necessary changes in the referenced device. (Emphasis added)

In the present case, no motivation is provided by any of the references to produce the present invention. Only by hindsight and with the knowledge of the present application could one reasonably propose that the cited prior art renders the invention obvious. Furthermore, as pointed out on numerous occasions by the Federal Circuit, the use of "hindsight" gleaned from the Appellants' specification is an entirely improper means for finding a motivation to combine cited references. *In re Corkill*, 226 U.S.P.Q. 1005, 1008 (CAFC 1985).

Even if the references are properly combinable, they nevertheless fail to teach or suggest the invention. As pointed out above, none of the references discloses the requisite combination of known start site and gene sequence. For the foregoing reasons, it is respectfully submitted that the invention as defined by claims currently in the case are patentable over the art.

4. Secondary Considerations

As the Court of Appeals for the Federal Circuit has unequivocally stated in *Custom Accessories, Inc. v. Jeffrey-Allan Industries, Inc.*, 1 U.S.P.Q.2d 1196 (Fed. Cir. 1986):

> Objective evidence of non-obviousness includes commercial success, long-felt but unresolved need, failure of others, and copying. When present, such objective evidence must be considered. It can be the most probative evidence of non-obviousness in the record, and enables the district court to avert the trap of hindsight. On the other hand, the absence of objective evidence does not preclude a holding of non-obviousness because such evidence is not a requirement for patentability.

These objective criteria are individually addressed below as they each apply to the present invention. The Board is respectfully requested to give all such evidence the requisite consideration.

Long-felt But Unresolved Need

The continuing long-felt and unresolved need in our communities for safe and effective means to eliminate and protect against the toxic effects of organophosphorus compounds is well known and has continued to be an issue at the forefront of public attention. This continuing and unresolved concern was poignantly and graphically demonstrated in at least two recent television broadcasts which are provided for the Board's review.

The sensitivity of certain individuals to home pest control application of organophosphorus pesticides such as dursban was the topic of a thirteen (13) minute segment of NBC Today on May 16, 1991 (Exhibit D). In this documentary (pp. 26-28) Katherine Couric, co-host, narrates the story of Mrs. Chris Weidner, an individual who suffers from a sensitivity to organophosphorus pesticides such as dursban. The graphic account related in this exhibit speaks for itself.

However, the Board's attention is particularly drawn to the discussion between Dr. Marion Moses of The Pesticide Education Center and Warren Stickle of the Chemical Producer's and Distributor's Association. Dr. Moses' comments are apparently much more in keeping with current EPA evaluations of these toxic compounds than are Mr. Stickle's. *See, e.g.*, Exhibits F-K which is a collection of "Pesticide Fact Sheets" published by the EPA, which fact sheets clearly show the concern which the government has concerning compounds such as ethyl parathion, Diazinon, fenitrothion, coumaphos, acephate and malathion.

Even more alarming was the report (Exhibit C) presented by Peter Jennings on ABC Wold News Tonight concerning Diazinon poisonings due to home applications, especially such exposures to small children, shown on May 9, 1991. In this documentary, reporter Bill Greenwood narrates the tragic Diazinon poisoning of Mr. Tom Latimer. Again, the Board's attention is drawn to the tragic consequences of such pesticide poisonings. The Board is provided a transcript of this program which is not as descriptive as the video tape itself. If so desired, the Appellants can produce the tape for viewing at the Board's convenience.

Protection systems for home applications of organophosphorus pesticides are certainly of paramount need and, as yet, remain an unresolved problem for modern science. The Board's attention is additionally directed to the desire by major producers of these pesticides to develop technology to degrade pesticides both at the production level and at the consumer level. The Board's attention is drawn to the March 4, 1995 letter to Ms. Ann Levy (whose company TECHSOURCE represented Appellants in licensing the present invention) from American

Cyanamid Co. (Exhibit N) and to the letter of June 5, 1991 to Dr. D. R. Eger (of the same firm) from the Ortho Division of the Chevron Chemical Co. (Exhibit L; follow-up letter at Exhibit M). These letters both further support the long and unsatisfied need in the commercial community for the products and processes of the present invention.

However, detoxification problems of startling proportions involving organophosphorus neurotoxin confront both government and commercial endeavors as well and are not limited to home pesticide use. The Board is reminded of the surprising finding by the present inventors that the enzyme of the present invention was effective in detoxifying one of the most common nerve agents produced by the military. The Board's attention is first drawn to a recent newspaper report by Keith Schneider of the New York Times published in the Houston Chronicle on Sunday, May 5, 1991 (Exhibit A, p. 12A). It will be noted in that article, that:

> Hampered by flaws in design and operation, the Army's program to incinerate the nation's enormous stockpile of chemical weapons is falling years behind schedule, experiencing huge cost increases and stirring public protests in six states and the South Pacific. . . . The project is part of the more than $200 billion the Pentagon has proposed to spend in the next 30 to 50 years to prevent the spread of poisons and clean up its contaminated bases and munitions factories . . . . [T]he Army has proposed burning all its mustard gas and nerve agents [primarily organophosphorus compounds] -- 60 million to 70 million pounds of the world's deadliest chemicals -- in nine incinerators.

As noted in the specification, p. 2, lines 1-5, the U.S. Army Research office provided funding for portions of the research involved in the making of this invention. More recently, the Department of the Navy has provided similar funding. While incineration has apparently been chosen as the most expeditious means of detoxifying these compounds, the Army maintains interest in using the enzyme, if they are produced on a commercially feasible scale, to handle

localized detoxification in spills, cleanups and transfer operations.

Commercial Success

Coupled with the clearly existing long-felt and unresolved needs established above is the initial proof of commercial success for the pilot-scale products and processes of the present invention. As evidence of this initial success, the Board's attention is drawn to the letter discussing a contractual agreement between the Chevron Chemical Company and the Assignee of the present invention (Exhibit M).

This Agreement notes that payment of $25,000 for evaluation of the products and process of the present invention is anticipated. That this commercial success has been a result of the nature and acceptance of the invention rather than from some relatively unrelated fact, such as marketing is most clearly indicated by the sophistication of the purchasing party, the Ortho Division of the Chevron Chemical Company. Since the market for this product is as yet undeveloped, no data can be presented as to market share, growth in market share, replacement of earlier products sold by others, or retail dollar amounts. However, the nexus required to exist between the claimed invention and the commercial success is evident.

Another indicia of the commercial success of the present invention is the apparent interest in supporting research and development of the recombinant enzyme by biotechnology companies such as Amgen. Cüneyt M. Serdar, the first author of Serdar et al. (1989), began research in this area under Douglas M. Munnecke at the University of Oklahoma (*see, e.g.*, Serdar et al.

[1982]), completed a PH.D. in this area at the University of Texas at Austin under David T. Gibson (*see, e.g.*, Serdar and Gibson [1985]) and apparently was encouraged to continue the work after he was hired by Amgen Inc. of Thousand Oaks, CA (*see, e.g.*, Serdar et al. [189]). This work was apparently carried out by Serdar at Amgen even in the face of all of the prior art cited by the Examiner in this Office Action and other prior art made of record in the Information Disclosure Statement filed in this case. Since it is very unlikely that a commercially successful biotechnology firm such as Amgen would fund research and development of a project that held no commercial potential for success, one may infer that at lest Amgen considers the development of the recombinant OPA enzyme to have a potential for commercial success.

Copying

> Copying the claimed invention, rather than one in the public domain, is indicative of non-obviousness. *Specialty Composites v. Cabot Corp.*, 6 U.S.P.Q.2d 1601 (Fed. Cir. 1988).

The Board's attention is drawn to the clear copying by others of the present invention. If the skilled artisan were to follow the teachings of the prior art, he would fail to derive the present invention. For instance, if the skilled artisan were to follow the teachings of either Serdar, et al. or Mulbry et al., he would isolate a very large segment of DNA from the active plasmid (in excess of 5 kb). He would then attempt to express this heterologously and fail (*see, e.g.*, both references.) He would, therefore, be unable to obtain purified OPA> Lacking the purified protein, he would have no way to determine the location of the opd gene. It is only by copying the methods of the represent invention that others were able to produce the sized-down

DNA fragment. In particular, if the skilled artisan were to combine the teachings of McDaniel et al. which incorrectly showed the position and nature of the start signal, he would fail. Only by coping the start signal and sequence disclosed by the present invention were others capable of repeating the invention.

Failure of Others

> The differences between the prior art and the invention defined by the asserted claims, the availability of that art to all workers int eh field, the failure of established competitors in a highly competitive market to make the invention despite the incentive to do so, the admittedly non-obvious performance benefits realized through the claimed invention, the impressive commercial success of the claimed product, the praise of independent commentators and the forbearance of competitors from infringing the patent all go to confirm the claimed invention was not obvious at the time it was made to a person of ordinary skill in the art. *S.C. Johnson & Son, Inc. v. Carter-Wallace, Inc.*, 225 U.S.P.Q. 1022 (N.Y. 1985).

Moreover, even the prior art cited by the Examiner shows the failures to achieve the present invention by others. The Board's attention has been drawn in arguments made above to numerous of these failures. However, in summary, it can be unequivocally stated that no other worker in the field, including the inventors themselves, had achieved the present invention until it was achieved by the inventors and disclosed in the present application. In fact, others had failed to:

(1) identify the actual start signal;

(2) sequence the gene;

(3) heterologously express sufficient quantities of OPA to allow determination of the amino acid sequence; or

(4) transform eukaryotic cell lines with opd.

These failures occurred even in the face of readily available prior art to all workers in the field,

and even in view of the impetus for established competitors in the highly competitive market to make the invention.

From the foregoing remarks, it is submitted that a conclusion of nonobviousness is compelled. The Appellant has addressed each of the Examiner's comments in the Office Action as they pertain to Section 103 rejections.

(v) Other Rejections

ISSUE I: Is Restriction to Claims 53-64 Proper?

Appellants agree with the appropriateness of the restriction requirement carried over from the parent case.

ISSUE II: Have Applicants Complied With 37 C.F.R. 1.52(c), 1.56 and 1.67(a)?

In their Response to the Official Action Mailed May 24, 1991, in the parent case Serial Number 07/344,258, Appellants responded to each of the Examiner's objections/rejection under these statutes. Specifically, the Appellants' attorney, who is also the principal inventor, at page 3, lines 16-27, stated:

> The Examiner also has required the cancellation of the alterations made in the originally filed specification which were initialed by one of the inventors but not dated. It was believed by the Applicants at the time of filing that initialling the non-substantive alterations was adequate to make the changes in the case. However, it is apparent that alteration cannot be made in such a manner and *these alterations are requested to be canceled according to the Examiner's requirements. The cancellation of these alterations is not believed to alter in any substantial manner the content or matter in the specification.* Amendments corresponding to the originally initialed but undated alterations have been made above.

The principal inventor is the prosecuting attorney. The statements made in the Response as noted above comply in every regard to the requirements of the cited statutes: alterations made after the signing of the declarations were canceled; a full explanation of the making of the alterations was provided. The Response was signed by C. Steven McDaniel in his capacity as

principal inventor, as the person who made the alterations and had direct knowledge of the timing and intent of the alterations, and as the prosecuting attorney of the case. Thus, there is no need for an additional affidavit merely repeating that which has already been averred by the attorney-appellant-inventor.

ISSUE III: Have Applicants Used Trademarks Inappropriately?

The Appellants have not used trademarks inappropriately, or if they have, such use was corrected in the parent case. In their Response to the Official Action Mailed May 24, 1991, in the parent case Serial Number 07/344,258, Appellants responded to each of the Examiner's objections/rejection under this argument. Specifically, at page 3, lines 28-32 through page 4, lines 1-20, Appellants corrected the three places in the specification that cited a trademark without reference to the company owning the rights in the mark. All other positions at which the Examiner noted unreferenced trademarks were reviewed but not altered as these were not uses of trademarks. Exhibits F-K are provided as additional indications that the common names of each of the OPs which the Examiner finds objectionable, are in fact, common names by which the OPs are referred.

CONCLUSION

The each of the bases for the Examiner's rejection of the claims on appeal have each been addressed. The Appellants maintain that they have invented a novel and unobvious recombinant OP-degrading enzyme. They also maintain that they have fully enabled, disclosed and taught the methods of using this recombinant enzyme to detoxify OPs. In particular, they maintain that they disclosed the best DNA sequence available at the time of filing of the application, and that although there may be minor modifications necessary in view of subsequently applied techniques, that the modifications requested were inherent in the molecule as was fully disclosed and enabled by the application. Much of the principal art cited by the Examiner is maintained by the Appellants to be removed by statements of the inventor/attorney who directed the work of non-inventor assistants. Other of the art cited is clearly a teaching away from the present invention. Even if there was a prima facie obviousness of the invention by the combinations proposed by the Examiner, the Appellants maintain that it is overcome by

a strong showing of secondary considerations. Therefore, the Appellants respectfully request the Board to reverse the Examiner's rejections and issue the present claims.

APPENDIX

Claims on Appeal

53. A method for detoxifying an organophosphorus compound comprising exposing said compound to recombinant bacterial organophosphorus acid anhydrase.

54. The method of claim 53 wherein said exposure is accomplished by passing said compound through a matrix comprising said recombinant anhydrase.

55. The method of claim 54 wherein said matrix is further comprised of a filtration device.

56. The method of claim 55 wherein said device is a gas mask.

57. The method of claim 53 wherein said organophosphorus compound is in air.

58. The method of claim 53 wherein said organophosphorus compound is in a fluid.

59. The method of claim 53 wherein said exposure is accomplished by spraying said recombinant anhydrase on a locus comprising the organophosphorus compound.

60. The method of claim 53 wherein said exposure is accomplished by introducing said anhydrase into a container comprising the organophosphorus compound.

61. The method of claim 53 wherein said recombinant bacterial organophosphorus acid anhydrase is produced by a transformed microorganism comprising an expression vector for producing said anhydrase and wherein said vector has a cloned bacterial organophosphorus acid anhydrase gene fragment with the DNA

coding sequence:

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5'
          CTGCAGCCTGACTCGGCACCAGTCGCTGCAAGCAGAGTCGTAAGCAATCGCAAGGGGGCAGC
ATG CAA ACG AGA AGG GTT GTG CTC AAG TCT GCG GCC GCA GGA ACT CTG CTC GGC
met gln thr arg arg val val leu lys ser ala ala ala gly thr leu leu gly

GGC CTG GCT GGG TGC GCG ACG TGG CTG GAT CGA TCG GCA CAG GCG ATC GGA TCA
gly leu ala gly cys ala thr trp leu asp arg ser ala gln ala ile gly ser

ATA CGT GCG CGT CCT ATC ACA ATC TCT GAA GCG GGT TTC ACA CTG ACT CAC GAG
ile arg ala arg pro ile thr ile ser glu ala gly phe thr leu thr his glu

GAC ATC TGC GGC AGC TCG GCA GGA TTC TTG CGT GCT TGG CCA GAG TTC TTC GGT
asp ile cys gly ser ser ala gly phe leu arg ala trp pro glu phe phe gly

AGC CGC AAA GCT CTA GCG GAA AAG GCT GTG AGA GGA TTG CGC GCC AGA GCG GCT
ser arg lys ala leu ala glu lys ala val arg gly leu arg ala arg ala ala

GGC GTG CGA ACG ATT GTC GAT GTG TCG ACT TTC GAT ATC GGT CGC GAC GTC AGT
gly val arg thr ile val asp val ser thr phe asp ile gly arg asp val ser

TTA TTG GCC GAG GTT TCG CGG GCT GCC GAC GTT CAT ATC GTG GCG GCG ACC GGC
leu leu ala glu val ser arg ala ala asp val his ile val ala ala thr gly

TTG TGG TTC GAC CCG CCA CTT TCG ATG CGA TTG AGG TAT GTA GAG GAA CTC ACA
leu trp phe asp pro pro leu ser met arg leu arg tyr val glu glu leu thr

CAG TTC TTC CTG CGT GAG ATT CAA TAT GGC ATC GAA GAC ACC GGA ATT AGG GCG
gln phe phe leu arg glu ile gln tyr gly ile glu asp thr gly ile arg ala

GGC ATT ATC AAG GTC GCG ACC ACA GGC AAG GCG ACC CCC TTT CAG GAG TTA GTG
gly ile ile lys val ala thr thr gly lys ala thr pro phe gln glu leu val

TTA AAG GCG GCC GCC CGG GCC AGC TTG GCC ACC GGT GTT CCG GTA ACC ACT CAC
leu lys ala ala ala arg ala ser leu ala thr gly val pro val thr thr his

ACG GCA GCA AGT CAG CGC GAT GGT GAG CGA GGC AGG CCG CCA TTT TTG AGT CCG
thr ala ala ser gln arg asp gly glu arg gly arg pro pro phe leu ser pro

AAG CTT GAG CCC TCA CGG GTT TGT ATT GGT CAC AGC GAT GAT ACT GAC GAT TTG
lys leu glu pro ser arg val cys ile gly his ser asp asp thr asp asp leu

AGC TAT CTC ACC GCC CTG CTG CGC GGA TAC CTC ATC GGT CTA GAC CAC ATC CCG
ser tyr leu thr ala leu leu arg gly tyr leu ile gly leu asp his ile pro

CAC AGT GCG ATT GGT CTA GAA GAT AAT GCG AGT GCA TCA CCG CTC CTG GGC ATC
his ser ala ile gly leu glu asp asn ala ser ala ser pro leu leu gly ile

CGT TCG TGG CAA ACA CGG GCT CTC TTG ATC AAG GCG CTC ATC GAC CAA GGC TAC
arg ser trp gln thr arg ala leu leu ile lys ala leu ile asp gln gly tyr

ATG AAA CAA ATC CTC GTT TCG AAT GAC TGG CTG TTC GGG TTT TCG AGC TAT GTC
met lys gln ile leu val ser asn asp trp leu phe gly phe ser ser tyr val

ACC AAC ATC ATG GAC GTG ATG GAT CGC GTG AAC CCC GAC GGG ATG GCC TTC ATT
thr asn ile met asp val met asp arg val asn pro asp gly met ala phe ile

CCA CTG AGA GTG ATC CCA TTC TAC GAG AGA AGG GCG TCC CAC AGG AAA CGC TGC
pro leu arg val ile pro phe tyr glu arg arg ala ser his arg lys arg cys
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CAG GCA TCA CTG TGA
gln ala ser leu  .
CTAACCCGGCGCGGTTCTGTGTCACCGACTTGCCGTGCATGACGCCATCTGGATCCTTCCACGCAGCGGCC
ACTATTCCCCGTCAAGATACCGAACGATGAAGTCGCGCATCGATCGATAGGCATCTTCAATGTGATCAGGG
CTGCCACCTCCAAAGCCGGTGGCCACCCCTGTCGATAGTCTTGAGGGACGGTAGCGACGACCGTGCTTTTC
GTGAACTGCAG
3.
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62. The method of claim 53 wherein said recombinant bacterial organophosphorus acid anhydrase is produced by a transformed eukaryotic cell line comprising an expression vector for producing said anhydrase and wherein said vector has a cloned bacterial organophosphorus acid anhydrase gene fragment with the DNA coding sequence:

```
5'
          CTGCAGCCTGACTCGGCACCAGTCGCTGCAAGCAGAGTCGTAAGCAATCGCAAGGGGGCAGC
ATG CAA ACG AGA AGG GTT GTG CTC AAG TCT GCG GCC GCA GGA ACT CTG CTC GGC
met gln thr arg arg val val leu lys ser ala ala ala gly thr leu leu gly

GGC CTG GCT GGG TGC GCG ACG TGG CTG GAT CGA TCG GCA CAG GCG ATC GGA TCA
gly leu ala gly cys ala thr trp leu asp arg ser ala gln ala ile gly ser

ATA CGT GCG CGT CCT ATC ACA ATC TCT GAA GCG GGT TTC ACA CTG ACT CAC GAG
ile arg ala arg pro ile thr ile ser glu ala gly phe thr leu thr his glu

GAC ATC TGC GGC AGC TCG GCA GGA TTC TTG CGT GCT TGG CCA GAG TTC TTC GGT
asp ile cys gly ser ser ala gly phe leu arg ala trp pro glu phe phe gly

AGC CGC AAA GCT CTA GCG GAA AAG GCT GTG AGA GGA TTG CGC GCC AGA GCG GCT
ser arg lys ala leu ala glu lys ala val arg gly leu arg ala arg ala ala

GGC GTG CGA ACG ATT GTC GAT GTG TCG ACT TTC GAT ATC GGT CGC GAC GTC AGT
gly val arg thr ile val asp val ser thr phe asp ile gly arg asp val ser

TTA TTG GCC GAG GTT TCG CGG GCT GCC GAC GTT CAT ATC GTG GCG GCG ACC GGC
leu leu ala glu val ser arg ala ala asp val his ile val ala ala thr gly

TTG TGG TTC GAC CCG CCA CTT TCG ATG CGA TTG AGG TAT GTA GAG GAA CTC ACA
leu trp phe asp pro pro leu ser met arg leu arg tyr val glu glu leu thr

CAG TTC TTC CTG CGT GAG ATT CAA TAT GGC ATC GAA GAC ACC GGA ATT AGG GCG
gln phe phe leu arg glu ile gln tyr gly ile glu asp thr gly ile arg ala

GGC ATT ATC AAG GTC GCG ACC ACA GGC AAG GCG ACC CCC TTT CAG GAG TTA GTG
gly ile ile lys val ala thr thr gly lys ala thr pro phe gln glu leu val

TTA AAG GCG GCC GCC CGG GCC AGC TTG GCC ACC GGT GTT CCG GTA ACC ACT CAC
leu lys ala ala ala arg ala ser leu ala thr gly val pro val thr thr his

ACG GCA GCA AGT CAG CGC GAT GGT GAG CGA GGC AGG CCG CCA TTT TTG AGT CCG
thr ala ala ser gln arg asp gly glu arg gly arg pro pro phe leu ser pro

AAG CTT GAG CCC TCA CGG GTT TGT ATT GGT CAC AGC GAT GAT ACT GAC GAT TTG
lys leu glu pro ser arg val cys ile gly his ser asp asp thr asp asp leu
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AGC TAT CTC ACC GCC CTG CTG CGC GGA TAC CTC ATC GGT CTA GAC CAC ATC CCG
ser tyr leu thr ala leu leu arg gly tyr leu ile gly leu asp his ile pro

CAC AGT GCG ATT GGT CTA GAA GAT AAT GCG AGT GCA TCA CCG CTC CTG GGC ATC
his ser ala ile gly leu glu asp asn ala ser ala ser pro leu leu gly ile

CGT TCG TGG CAA ACA CGG GCT CTC TTG ATC AAG GCG CTC ATC GAC CAA GGC TAC
arg ser trp gln thr arg ala leu leu ile lys ala leu ile asp gln gly tyr

ATG AAA CAA ATC CTC GTT TCG AAT GAC TGG CTG TTC GGG TTT TCG AGC TAT GTC
met lys gln ile leu val ser asn asp trp leu phe gly phe ser ser tyr val

ACC AAC ATC ATG GAC GTG ATG GAT CGC GTG AAC CCC GAC GGG ATG GCC TTC ATT
thr asn ile met asp val met asp arg val asn pro asp gly met ala phe ile

CCA CTG AGA GTG ATC CCA TTC TAC GAG AGA AGG GCG TCC CAC AGG AAA CGC TGC
pro leu arg val ile pro phe tyr glu arg arg ala ser his arg lys arg cys

CAG GCA TCA CTG TGA
gln ala ser leu  .
CTAACCCGGCGCGGTTCTGTGTCACCGACTTGCCGTGCATGACGCCATCTGGATCCTTCCACGCAGCGGCC
ACTATTCCCCGTCAAGATACCGAACGATGAAGTCGCGCATCGATCGATAGGCATCTTCAATGTGATCAGGG
CTGCCACCTCCAAAGCCGGTGGCCACCCCTGTCGATAGTCTTGAGGGACGGTAGCGACGACCGTGCTTTTC
GTGAACTGCAG
3.
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63. The method of claim 53 wherein said recombinant bacterial organophosphorus acid anhydrase is produced by a transformed eukaryotic organism comprising an expression vector for producing said anhydrase and wherein said vector has a cloned bacterial organophosphorus acid anhydrase gene fragment with the DNA coding sequence:

```
5'
        CTGCAGCCTGACTCGGCACCAGTCGCTGCAAGCAGAGTCGTAAGCAATCGCAAGGGGGCAGC
ATG CAA ACG AGA AGG GTT GTG CTC AAG TCT GCG GCC GCA GGA ACT CTG CTC GGC
met gln thr arg arg val val leu lys ser ala ala ala gly thr leu leu gly

GGC CTG GCT GGG TGC GCG ACG TGG CTG GAT CGA TCG GCA CAG GCG ATC GGA TCA
gly leu ala gly cys ala thr trp leu asp arg ser ala gln ala ile gly ser

ATA CGT GCG CGT CCT ATC ACA ATC TCT GAA GCG GGT TTC ACA CTG ACT CAC GAG
ile arg ala arg pro ile thr ile ser glu ala gly phe thr leu thr his glu

GAC ATC TGC GGC AGC TCG GCA GGA TTC TTG CGT GCT TGG CCA GAG TTC TTC GGT
asp ile cys gly ser ser ala gly phe leu arg ala trp pro glu phe phe gly

AGC CGC AAA GCT CTA GCG GAA AAG GCT GTG AGA GGA TTG CGC GCC AGA GCG GCT
ser arg lys ala leu ala glu lys ala val arg gly leu arg ala arg ala ala

GGC GTG CGA ACG ATT GTC GAT GTG TCG ACT TTC GAT ATC GGT CGC GAC GTC AGT
gly val arg thr ile val asp val ser thr phe asp ile gly arg asp val ser

TTA TTG GCC GAG GTT TCG CGG GCT GCC GAC GTT CAT ATC GTG GCG GCG ACC GGC
leu leu ala glu val ser arg ala ala asp val his ile val ala ala thr gly
```

```
TTG TGG TTC GAC CCG CCA CTT TCG ATG CGA TTG AGG TAT GTA GAG GAA CTC ACA
leu trp phe asp pro pro leu ser met arg leu arg tyr val glu glu leu thr

CAG TTC TTC CTG CGT GAG ATT CAA TAT GGC ATC GAA GAC ACC GGA ATT AGG GCG
gln phe phe leu arg glu ile gln tyr gly ile glu asp thr gly ile arg ala

GGC ATT ATC AAG GTC GCG ACC ACA GGC AAG GCG ACC CCC TTT CAG GAG TTA GTG
gly ile ile lys val ala thr thr gly lys ala thr pro phe gln glu leu val

TTA AAG GCG GCC GCC CGG GCC AGC TTG GCC ACC GGT GTT CCG GTA ACC ACT CAC
leu lys ala ala ala arg ala ser leu ala thr gly val pro val thr thr his

ACG GCA GCA AGT CAG CGC GAT GGT GAG CGA GGC AGG CCG CCA TTT TTG AGT CCG
thr ala ala ser gln arg asp gly glu arg gly arg pro pro phe leu ser pro

AAG CTT GAG CCC TCA CGG GTT TGT ATT GGT CAC AGC GAT GAT ACT GAC GAT TTG
lys leu glu pro ser arg val cys ile gly his ser asp asp thr asp asp leu

AGC TAT CTC ACC GCC CTG CTG CGC GGA TAC CTC ATC GGT CTA GAC CAC ATC CCG
ser tyr leu thr ala leu leu arg gly tyr leu ile gly leu asp his ile pro

CAC AGT GCG ATT GGT CTA GAA GAT AAT GCG AGT GCA TCA CCG CTC CTG GGC ATC
his ser ala ile gly leu glu asp asn ala ser ala ser pro leu leu gly ile

CGT TCG TGG CAA ACA CGG GCT CTC TTG ATC AAG GCG CTC ATC GAC CAA GGC TAC
arg ser trp gln thr arg ala leu leu ile lys ala leu ile asp gln gly tyr

ATG AAA CAA ATC CTC GTT TCG AAT GAC TGG CTG TTC GGG TTT TCG AGC TAT GTC
met lys gln ile leu val ser asn asp trp leu phe gly phe ser ser tyr val

ACC AAC ATC ATG GAC GTG ATG GAT CGC GTG AAC CCC GAC GGG ATG GCC TTC ATT
thr asn ile met asp val met asp arg val asn pro asp gly met ala phe ile

CCA CTG AGA GTG ATC CCA TTC TAC GAG AGA AGG GCG TCC CAC AGG AAA CGC TGC
pro leu arg val ile pro phe tyr glu arg arg ala ser his arg lys arg cys

CAG GCA TCA CTG TGA
gln ala ser leu  .
CTAACCCGGCGCGGTTCTGTGTCACCGACTTGCCGTGCATGACGCCATCTGGATCCTTCCACGCAGCGGCC
ACTATTCCCCGTCAAGATACCGAACGATGAAGTCGCGCATCGATCGATAGGCATCTTCAATGTGATCAGGG
CTGCCACCTCCAAAGCCGGTGGCCACCCCTGTCGATAGTCTTGAGGGACGGTAGCGACGACCGTGCTTTTC
GTGAACTGCAG
3.
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Table of Authorities

Exhibits List